THE

RICE INSTITUTE PAMPHLET

ADDRESS BY DR. PAUL F. RUSSELL

and

SYMPOSIUM

on

RESISTANCE AND IMMUNITY IN PARASITIC INFECTIONS

Volume XLV April, 1958 Number 1

THE RICE INSTITUTE PAMPHLET *is published at Houston, Texas, and is issued in January, April, July, and October. It was entered as second-class matter, April 15, 1915, at the post office at Houston, Texas, under the act of August 24, 1912.*

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vol. 45

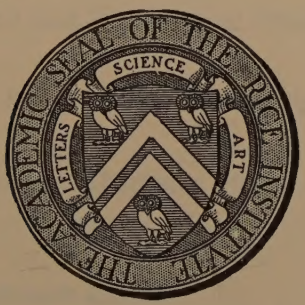
THE RICE INSTITUTE PAMPHLET

Vol. XLV April, 1958 No. 1

PROGRAM IN CELEBRATION OF THE OPENING OF

THE M. D. ANDERSON BIOLOGICAL LABORATORIES

Thursday, January 30 to Saturday, February 1, 1958



Published by

THE RICE INSTITUTE

a university of liberal and technical learning
founded by William Marsh Rice in the City
of Houston, Texas, and dedicated by him to
the advancement of Letters, Science, and Art

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CONTENTS

	Pages
WILLIAM V. HOUSTON Address of Welcome	1- 3
ASA C. CHANDLER Introductory Remarks	4- 8
PAUL F. RUSSELL Man Against Malaria—Progress and Problems	9- 22
CECIL A. HOARE The Enigma of Host-Parasite Relations in Amebiasis	23- 35
CLARK P. READ Status of Behavioral and Physiological "Resistance"	36- 54
CLAY G. HUFF Host Influences on Some Haemosporidian Parasites	55- 79
LESLIE A. STAUBER Host Resistance to the Khartoum Strain of *Leishmania donovani*	80- 96
ROBERT M. LEWERT Invasiveness of Helminth *Larvae*	97-113
WILLIAM H. TALIAFERRO The Synthesis and Activities of Antibodies	114-140
JOSÉ OLIVER GONZALEZ AND ENRIQUE KOPPISCH Immunological and Pathological Phenomena Related to Substances from Tissues of *Ascaris lumbricoides*	141-150
IRVING G. KAGAN Contributions to the Immunology and Serology of Schistosomiasis	151-183
NORMAN R. STOLL The Induction of Self-cure and Protection with Special Reference to Experimental Vaccination Against *Haemonchus*	184-208

ADDRESS OF WELCOME

WILLIAM V. HOUSTON
President of the Rice Institute

IT GIVES me great pleasure to express the welcome of the Rice Institute to all of you here to help us celebrate the opening of our new biology building. For many of you this may be your first visit to the Rice Institute, and I hope we may have an opportunity to make you somewhat acquainted with our facilities and our activities. We are also happy to welcome back so many of our former graduate students; many of you, I am sure, will be pleasantly surprised to see the changes that have taken place on our campus since you were here before.

The name "Institute" is sometimes misleading, since we are not an institute of technology. We aim to be a small university, small in total numbers of people and small in that we confine our efforts to restricted fields largely of the traditional university variety. We are firmly dedicated to the proposition that size and excellence are not at all synonymous. In fact, we believe that we can pursue excellence better in a small institution than some can in institutions much larger.

I have recently come across what appears to me to be an interesting item concerning the early history of the Rice Institute. When Dr. Lovett was appointed the first President, in December, 1907, he resolved to take a trip around the world to view universities in foreign lands as well as in his country. At that time there seems to have been considerable interest in a Russian system of technical institutes. Consequently, he made a visit to Russia, and, in fact, went clear across Asia on the Trans-Siberian Railway to Japan, after viewing a number of Russian schools. His conclusion at that time was that he did not want to develop the Rice Institute along the Russian lines, but proposed instead to develop along the lines of more traditional Western European and American universities. Since Dr. Lovett was coming from Princeton, it was inevitable that many features of the Rice Institute should be similar to corresponding features at Princeton, and I believe the Princeton tradition is still strong among us. Whether our current interest in Russian education should lead us to the same conclusion, I am not now prepared to say, but I find this an interesting recurrence of an historical situation.

The principal way in which we cultivate our university nature is our emphasis on graduate work. We do graduate work to the Doctor's degree in most departments, although here again we try to be very careful that

graduate work is undertaken only when we have adequate facilities and adequate faculty, and to my mind the adequacy of the faculty is much the more important feature. We have been doing graduate work in biology for some thirty years. I was very happy, at a homecoming session last night, to welcome back so many of our former biology graduate students, many of whom have since had distinguished careers. Many of you I was enabled to meet for the first time, since compared with Professor Chandler I am a very recent newcomer.

Another of our features in which some of you may be interested is our newly inaugurated set of residential colleges. Our dinner last night was held in Baker College, and the dinner this evening is to be held in Wiess College. These colleges are not, of course, particularly connected with our program of graduate studies and research, but they are devised somewhat along the lines of the colleges at Yale, the houses at the California Institute of Technology, and remotely the colleges in Oxford and Cambridge, to provide an organization of undergraduate life most conducive to educational objectives. Dr. Talmage, Chairman of our Biology Department, is the Master of Weiss College, and has been active and intimately associated with the inauguration of this new activity.

This symposium, as well as yesterday evening's homecoming, has been arranged with two objectives in mind. The first, of course, is to show off the new building. The M. D. Anderson Biological Laboratories have been built with the aid of the M. D. Anderson Foundation. This Foundation, established by Monroe D. Anderson of this city, who died a number of years ago, has been one of the most active in the development of the Texas Medical Center and in the promotion of other worthwhile activities in this city. In particular, we have on our campus Anderson Hall, which houses the offices of many of our faculty in English, history, mathematics, languages, and architecture, and there is in the Medical Center the M. D. Anderson Cancer Research Hospital, of which I am sure many of you have heard. These are only some of the benefactions and contributions to Houston's development that are due to the Anderson Foundation.

Of course, a building is not the only thing. The building needs equipment, and we are happy to acknowledge the fact that adequate equipment for the research activities has been made possible because of a grant from the National Institutes of Health. Because of this grant, which is, of course, matched by similar funds from the Rice Institute, we shall be able to equip the building with research facilities much more satisfactorily than might otherwise have been the case.

On the other and, I think all of us here realize, and I would like to reiterate, that a building and its equipment constitute only an inert skeleton

for teaching and research in biology or anything else. There must be flesh and blood, muscles, nerves, and above all, brains. We can hope that these physical facilities will help us in our continuing effort to add to the Rice Institute faculty men whose creative abilities, whose intense interest, and whose human qualities will make them outstanding in the university community in the next fifty years.

I will always remember a comment once made by Linus Pauling in my hearing. He said that the last paper of many a distinguished chemist was entitled "The Plans for My New Building." Perhaps by sufficient emphasis on the fact that something needs to be done *in* a new building we can avoid falling into such a trap.

The second object in arranging this symposium is to pay honor, before his retirement, to Professor A. C. Chandler. Professor Chandler has been on the faculty of the Rice Institute since 1919, and during that time his energy, his spirit, have been the driving force behind our activities. He is not yet retired, and will not retire for several years. However, it is our custom to rotate the chairmanships of departments, and, in particular, to ask a chairman to retire from that particular position some five years before his official retirement date. One of the objectives of such a scheme, in my mind, is to emphasize our regard for the professorship, rather than the administrative chairmanship, as the distinguished position. Somebody has to do some administering. It is a time-consuming and nerve-racking operation, and its function is to make it possible for other members of the faculty to do their jobs of teaching and research in the most effective way. This chore Professor Talmage has taken over for the past two years, leaving Professor Chandler free to devote himself to his teaching and research activities. Because of Professor Chandler's long and distinguished career here it has seemed quite appropriate that this symposium should be on the subject to which he has given his life's attention.

Again let me welcome all of you here today and to thank you for joining with us in these two objectives.

INTRODUCTORY REMARKS

ASA C. CHANDLER
The Rice Institute

Ladies and Gentlemen,

IT IS with very great pleasure that we welcome you here today. We are delighted to greet many old and distinguished friends who have come to help us celebrate an event that is at once a monument to our past efforts in biological teaching and research, and a challenge to still greater efforts in the future: the opening—or at least the approaching opening—of our new Biology Building. To us this new building is a veritable dream come true. As one of our symposium speakers very cleverly put it, the present symposium serves as the background music to accompany our being carried by our generous benefactors over the threshold of our new home.

The Rice Institute opened its doors in 1912, as an institution dedicated to the advancement of *Letters, Science and Art*—a monument to the generosity and foresightedness of one of Houston's most prominent pioneers, Mr. William Marsh Rice. The Biology Department had its beginning in the following year, when our first Professor of Biology, Dr. Julian Huxley, gave a freshman course to a class of 31 students. By 1915 our Biology staff had grown to three, including Dr. H. J. Muller, subsequently, as you know, a Nobel Prize winner in genetics. Dr. Huxley left in 1916 to serve his own country in her hour of need during World War I, and was replaced by Dr. Edgar Altenburg, who has had a long and distinguished career in genetics, and whom we still enjoy as a colleague. In 1919 I came here to replace Dr. Muller and, except for temporary sojourns in India and Egypt, have been here ever since. While I was in India Dr. R. P. Hall joined our staff, and while I was in Egypt we were honored by the presence of Dr. George R. LaRue. In 1928 Dr. M. A. Stewart, now a dean at the University of California in Berkeley, was added to our staff. We continued with a staff of three for a long time; this included at one time or another Dr. I. C. Kitchen, Dr. E. S. Deevey and Dr. E. J. Eversole. In 1940 Dr. J. I. Davies, who had come over with Dr. Huxley and had been an assistant, became a fourth member of the staff. He is still one of us and is with us today.

During this period we had only from two to four graduate students at a time, but many of these have subsequently distinguished themselves and are well known to the majority of the parasitologists in this audience. One

of these, our first, Dr. Paul D. Harwood, will preside at our session this afternoon. I also wish to pay tribute to Mrs. Evelyn Hake, my research assistant since 1931; without her clever technical help our output of work in parasitology and immunology would inevitably have been far less, as I know our former graduate students, here today to join us on this auspicious occasion, will whole-heartedly agree.

After World War II, when Dr. Houston joined us as our second President, and when the foresightedness of our trustees and the generosity of various benefactors made it financially possible, we began an expansion which has not yet run its full course. Our present highly efficient and progressive department chairman, Dr. Roy V. Talmage, endocrinologist, joined us in 1947; Dr. Jack Daugherty, whose work on physiology of parasites is well known to you, came a year or two later, then Dr. Allen Enders, primarily an histochemist, and, latest, Dr. Jorge Awapara, a biochemist. In addition, Dr. Hugh C. Welch has for many years been a lecturer in biology for our Physical Education division. In the not too distant future we hope to add one more member, in the field of microbiology. Meanwhile our number of graduate students has increased to a dozen or more, plus one or two postdoctoral fellows, and we average something like twenty published research papers a year.

With this expansion our work in our old quarters has been seriously hampered by lack of space. Our situation has been worse than that of sardines in a can, for there each sardine at least has a place to sit tight and call his own. But we couldn't do that—we have one laboratory in our old building in which five different lab courses are given on five successive days of the week. When we get in our new quarters I hope we don't explode too badly from sudden release of pressure!

In our new building we shall be able to accommodate a number of postdoctoral fellows and twenty or twenty-five graduate students. We anticipate that our new building will stimulate greatly the research program in our department. While a broad and diversified undergraduate program is planned, it is expected that the research endeavors will be restricted to complementing fields because of the modern demands for teamwork in research. Prime emphasis will be placed on the physiological sciences, genetics, microbiology, parasitology, and related fields. We expect that within a few years even the greatly expanded facilities in our new home will be stretched to capacity, for the very availability of these facilities and our enlarged staff will attract more graduate students and increase our research activity.

The present symposium, as you all recognize, is in the field of work in which I and my graduate students have been particularly interested for the past twenty-five or thirty years. I wish to take this occasion to

express my deep appreciation for this tribute on the part of President Houston and of my colleagues in the Biology Department. It is a gratifying recognition of the contributions we have made in this field.

Our work on resistance and immunity began with a series of experiments, published in 1932, on the nature of the resistance of rats to superinfection with the nematode, *Nippostrongylus muris*. A year prior to this Dr. Schwartz, who is honoring us with his presence here today, and his colleagues had already demonstrated (Schwartz *et al.*, 1931), in a paper that at the time escaped our attention, that after a single infection with this nematode, and increasingly with subsequent infections, the host built up a resistance that manifests itself by retardation of growth, development, and reproduction, and postulated the development by the host of a specific growth-inhibiting substance or substances. They also expressed the opinion that a similar mechanism of resistance would be found in other nematode infections. Here at Rice Institute we continued work on the details of this newly demonstrated type of resistance in a series of papers published from 1935 to 1938. In 1935 (Chandler, 1935) we came to the conclusion that many of the phenomena manifested in superinfections—stunting in growth, retardation of development, inhibition of reproduction, and failure of successful migration through the body—might be the result of interference with nutrition, and might be due to the development of anti-enzymes which inhibit the activity of the enzymes by means of which the parasites digest and assimilate the host's protein. We felt therefore that immunity to helminthic infections was due to an antibody reaction, not to the body substance of the worms, but to metabolic products—excretions or secretions; and we suggested that the inhibition of reproduction of *Trypanosoma lewisi*, which Dr. Taliaferro and his associates had demonstrated in a brilliant series of researches (Taliaferro, 1932), might be a similar phenomenon. We feel that we have recently demonstrated (Thillet and Chandler, 1957), at least to our satisfaction, that this surmise was correct, and that the action of ablastin is in every way comparable to the inhibition of growth, development and reproduction in Nippostronglyus and other parasitic nematodes. In the latter case the demonstration by Sarles and Taliaferro of precipitates in excretions and in the intestine of Nippostrongylus larvae immersed in immune serum confirmed our ideas. Since then a similar phenomenon has been demonstrated in numerous nematodes, as well as in schistosome cercariae. In more recent years direct evidence for the role of excretions and secretions of helminths in stimulating an immune response in the host, which Chipman in her 1957 paper referred to as a generally accepted view, has been provided by Thorson (1953) for *Nippostrongylus*

by Thorson (1956) for *Ancylostoma caninum;* by Campbell (1955), Chute (1956) and Chipman (1957) for *Trichinella;* and by Levine for *Schistosoma mansoni* (quoted by Kagan, 1958).

In 1939 and 1940 we began work on immunity in *Hymenolepis diminuta* infections (Chandler, 1939, 1940), in which evidence was adduced against any antibody response to these inhabitants of the intestinal lumen; this was extended to the acanthocephalan, *Moniliformis dubius,* by Burlingame (1941). In 1952-1954 some very interesting observations were made by Heyneman (1954) on immunity in *Hymenolepis* infections, particularly with reference to the difference in the course of *Hymenolepis nana* infections when induced by feeding of eggs, thus involving a parenteral phase when the cysticercoids develop in the villi, and when induced by beetle-reared cysticerci when the infection is confined to the intestinal lumen. A particularly interesting observation was the fact that lumen-dwelling worms, either *H. diminuta* or *H. nana,* though incapable of stimulating immunity, were affected to some degree by the immunity resulting from prior development of *H. nana* cysticercoids in the villi.

We have played around with immunity in many other infections, including work by Dr. Schuhardt (Schuhardt and Wilkerson, 1951) on spirochetes, though this was done after he left the Institute; by Dr. Thillet (Thillet and Chandler, 1957) on *Trypanosoma lewisi;* by Dr. Healy (1955) on *Fasciola* infections; and by Mr. Esslinger, presently working on screwworms. These men, too, are in our audience today. We are presently working also on immunological phenomena in *Entamoeba* and *Trichomonas* infections. An important factor in making our contributions to parasite immunology possible has been, and is, the series of generous research grants that we have received from the National Institutes of Health. It is a pleasure to acknowledge this aid and publicly express our appreciation before this audience.

From what I have said you will readily understand why we are so pleased to have the whole subject of resistance and immunity in parasitic infections discussed here today and tomorrow by the leaders in this field of work, and also why we are so pleased with the response to our invitations by fellow workers in all parts of our country. This symposium is a very great treat for us, and we hope it will be for you.

REFERENCES

Burlingame, P. L. and Chandler, A. C. 1941. Host-parasite relations of *Moniliformis dubius* (Acanthocephala) in albino rats and the environmental nature of resistance to single and superimposed infections with this parasite. *Am. J. Hyg.* **33,** Sect. D, 1-21.

Chandler, A. C. 1932. Experiments on the resistance of rats to superinfection with the nematode, *Nippostrongylus muris. Am. J. Hyg.* **16,** 750-782.

Chandler, A. C. 1935. Studies on the nature of immunity to intestinal helminths. *Am. J. Hyg.* **22,** 157-168.

Chandler, A. C. 1939. The effects of number and age of worms on development of primary and secondary infections with *Hymenolepis diminuta* in rats, and an investigation into the true nature of "premunition" in tapeworm infections. *Am. J. Hyg.* **29,** 105-114.

Chandler, A. C. 1940. Failure of artificial immunization to influence *Hymenolepis diminuta* infections in rats. *Am. J. Hyg.* **31,** Sect. D, 17-22.

Campbell, C. H. 1955. The antigenic role of the excretions and secretions of *Trichinella spiralis* in the production of immunity in mice. *J. Parasitol.* **41,** 483-491.

Chipman, P. B. 1957. The antigenic role of the excretions and secretions of adult *Trichinella spiralis* in the production of immunity in mice. *J. Parasitol.* **43,** 593-598.

Chute, R. M. 1956. The dual antibody response to experimental trichinosis. *Proc. Helm. Soc. Wash.* **23,** 49-58.

Healy, G. R. 1955. Studies on immunity to *Fasciola hepatica* in rabbits. *J. Parasitol.* **41,** Suppl., 35.

Heyneman, D. L. 1954. Studies on immunity induced by the dwarf mouse tapeworm, *Hymenolepis nana,* in white mice and rats. Ph.D. Thesis, Rice Institute.

Kagan, I. G. 1958. Contributions to the immunology and serology of schistosomiasis. *Rice Inst. Pamphlet* **45,** No. 1 (this issue).

Schuhardt, V. T. and Wilkerson, M. 1951. Relapse phenomena in rats infected with single spirochetes (*Borrelia recurrentis* var. *turicatae. J. Bacteriol.* **62,** 215-219.

Schwartz, B., Alicata, J. E. and Lucker, J. T. 1931. Resistance of rats to superinfection with a nematode, *Nippostrongylus muris,* and an apparently similar resistance of horses to superinfection with nematodes. *J. Wash. Acad. Sci.* **21,** 259-261.

Taliaferro, W. H. 1932. Trypanocidal and reproduction-inhibiting antibodies to *Trypanosoma lewisi* in rats and rabbits. *Am. J. Hyg.* **16,** 32-84.

Thillet, C. J., Jr. and Chandler, A. C. 1957. Immunization against *Trypanosoma lewisi* in rats by injections of metabolic products. *Science* **125,** 346-347.

Thorson, R. E. 1953. Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris. Am. J. Hyg.* **58,** 1-15.

Thorson, R. E. 1956. Proteolytic activity in extracts of the esophagus of adults of *Ancylostoma caninum* and the effect of immune serum on this activity. *J. Parasitol.* **42,** 21-25.

MAN AGAINST MALARIA—PROGRESS AND PROBLEMS

PAUL F. RUSSELL
The Rockefeller Foundation

PRESIDENT EISENHOWER, in his recent State of the Union message, declared that we now have it within our power to eradicate malaria, "the scourge of mankind," from the face of the earth. He called on the Soviets to join us in this great work of humanity which he cited as an example of "cooperation on projects of human welfare"—works of peace.

Surely, it is remarkable that in a country where malaria is a rare disease the President should give so much prominence to malaria eradication in a speech devoted to measures which he was recommending to the Congress in the interests of our own security. Remarkable, indeed, but logical in the light of present progress and problems in the age-old conflict between man and malaria, which is the subject of this address.

It seems to me very important that there should be widespread understanding of the history and present status of this insidious but devastating disease. Here in our own United States we now have so little direct evidence of malaria's power for evil that we tend to underestimate it and to forget its indirect damage to us. We fail to grasp the significance of a disease that in 1957 brought to an estimated 200 million persons, attacks of chills, fever, and debility, from the effects of which some two millions died. Who can evaluate the impact on the physical, social, and political health of the world of a disease that annually brings bodily weakness to approximately the same number of people as live in the United States, Canada, and Mexico combined?

Certainly, such an evaluation is not easily made by Americans, to most of whom this disease is practically unknown. For example, provisional data indicate that the remarkably low number of 144 malaria cases was reported to our National Office of Vital Statistics in 1957. The Public Health Service has surveyed 40 of these reports, confirming 24, of which only eight were found to represent infections contracted within the United States, four in California and four in Oklahoma (Dunn, 1957).

All but a few "old timers" have forgotten that malaria was once highly prevalent in this country. Moreover, only a few observers with special knowledge realize that although less than a dozen persons are known to

have contracted malaria in this country last year, yet during the same 12 months the disease probably cost us more than a third of a billion dollars, through higher priced imports and decreased exports, in our dealings with malarious countries.

So I thought it might be interesting to look for a few moments at malaria as once it prevailed here and in certain other areas around the world.

Malaria Once Prevalent in the United States

Just twenty years ago Williams (1938) one of the world's leading malariologists, estimated that the average incidence of malaria in the United States was four million cases a year, with as many as six or seven million afflicted in peak years such as 1935. Williams further stated that the average cost of malaria to the Southern United States was probably over half a billion dollars annually. This was the incidence and the economic importance of malaria after 30 years of active malaria control had greatly improved the situation.

In the 19th century, malaria was so common throughout the Mississippi Valley and much of the South that it was often accepted as a normal and inevitable part of life. Ague, *i.e.* chills and fever, as Ackerknecht (1947) has pointed out, although objectively dangerous and burdensome, had subjectively by social convention come to lose the character of a disease. "He ain't sick, he's only got the ager," was a typical comment of the period. For example, Petersen (1938) quotes an immigrant on the Mississippi in the 1830's, who wrote: "As we drew near Burlington [Iowa] in front of a little hut on the river bank, sat a girl and a lad—most pitiable looking objects, uncared for, hollow-eyed, sallow-faced. They had crawled out into the warm sun with chattering teeth to see the boat pass. To mother's inquiries the captain said: 'If you've never seen that kind of sickness I reckon you must be a Yankee; that's the ague. I'm feared you'll see plenty of it if you stay long in these parts. They call it here the swamp devil, and it will take the roses out of the cheeks of those plump little ones of yours mighty quick. Cure it? No, madam. No cure for it; have to wear it out. I had it a year when I first went on the river!"

This Mississippi Valley malaria made a deep impression on Charles Dickens when he traveled from Cincinnati to Saint Louis by river boat in 1842. In his *American Notes* he wrote of the place where the Ohio joins the Great River as follows: "At the junction of the two rivers, on ground so flat and low and marshy, that at certain seasons of the year it is inundated to the house-tops, lies a breeding place of fever, ague, and death, . . . a dismal swamp, . . . with rank unwholesome vegetation, in whose baleful shade the wretched wanderers who are tempted hither, droop, and die, and lay their bones." Later, in his novel *Martin Chuzzle-*

wit, Dickens described this area as "a marsh on which the good growth of the earth seemed to have been wrecked and cast away, that from its decomposing ashes vile and ugly things might rise; . . . where fatal maladies, seeking whom they might infect, came forth at night in misty shapes, and creeping out upon the water, hunted them like spectres until the day. . . ."

On his river trip, Dickens' face and nose were "profusely ornamented with the stings of mosquitoes" but, of course, he did not equate, as we do, the insects and the misty spectres rising from the marshes "seeking whom they might infect." Fortunately for Dickens his journey was made before the beginning of the malaria transmission season. The ague cases he saw and described were no doubt relapsing vivax infections.

Parenthetically, another aspect of American life that greatly impressed Dickens was the widespread use of chewing tobacco, that weed to which innumerable healing powers were once attributed, the *herba panacea* of the 16th century, the chewing of which, an Alabama farmer once told me, had given him freedom from chills and fever for many a year. In Washington, which Dickens considered a very unhealthy city, he noted in the Senate, "many honourable members with swelled faces." He discovered "that this appearance is caused by the quantity of tobacco they contrive to stow within the hollow of the cheek." Said Dickens: "It is strange enough too, to see an honourable gentleman leaning back in his tilted chair with his legs on the desk before him, shaping a convenient 'plug' with his penknife, and when it is quite ready for use, shooting the old one from his mouth, as from a popgun, and clapping the new one in its place." Dickens added that he "was surprised to observe that even steady old chewers of great experience, are not always good marksmen."

Not even Yankees in the 19th century were exempt from malaria. For instance, the late Alan Gregg (1956) wrote that his parents left Connecticut in 1882 for Colorado because of the frequency of malaria around their home in Hartford. Indeed, throughout the length and breadth of our country and spilling up into southern Canada, malaria in the 1800's was the most common infectious disease. Quinine in large bottles stood on the clock shelf of countless homes, together with a tumbler of water containing slippery elm bark, used as a "chaser." Barber (1929) noted that the taking of quinine for ague was a daily ritual—but not for prophylaxis, because the people were so rarely free from malaria that there was no question of anything but trying to cure it.

John Macculloch, an English physician (1829, p. 190) discussing those fevers in the United States which he believed were due to the noxious miasm he called "malaria," wrote as follows: "What the fate of much of this new country may ultimately be in this respect, it is difficult to foresee,

when we reflect on the numerous circumstances already noted, which modify the production and propagation of Malaria, and where so much is yet to be done as to alteration; though it is to be suspected that no changes and no cultivation will ever bring into a state of salubrity, a country so abounding with alluvial plains, even in the interior, and so extensively the produce of its numerous and enormous rivers."

Going back to Colonial times, we are uncertain whether or not malaria was indigenous in the Americas before the arrival of Europeans. But the disease undoubtedly became very common in the early settlements, in fact malaria not only plagued Colonial America but it also significantly delayed its development. The recurring spring and fall epidemics brought anemia and disability that seriously depleted the supply of labor. As expressed in a Boston news report of September, 1690 (Duffy, 1953) "Epidemical Fevers and Agues grow very common in some parts of the Country, whereof, tho' many dye not, yet they are sorely unfitted for their imployments." Another testimony is that of a Pennsylvania missionary in 1771 explaining why church construction in his parish was so slow: "In the beginning of last September the workmen fell sick of intermitting fevers, the epidemical distemper of this country, and the autumnal season was so far advanced before they recovered, that the utmost they could do was lay the floor" (Duffy, 1953).

One is tempted to speculate as to how different our history might have been had this country not been severely handicapped by malaria and other now preventable fevers during so much of its developmental period. So, too, we sometimes wonder how any progress at all was made in those days, or today in malarious underdeveloped countries, when we realize that to the now preventable fevers were added practically all of our own contemporary ills that press, radio, and T-V will not let us forget by day or by night.

Malaria in Europe

In Europe, in the last century, no region was exempt from malaria, not even Scandinavia, where there were serious epidemics from time to time, the last one in Finland in 1902. As recently as 1920-21, a malaria pandemic swept northwards through Russia even to the 64th degree of latitude, not far from Archangel (Hackett, 1949). Southern European countries have for centuries been greatly affected. Macculloch (1829, pp. 7-8) described the malaria blight very clearly in the following quotation: "Let us turn to Italy: the fairest portions of this fair land are a prey to this invisible enemy, its fragrant breezes are poison, the dews of its summer evenings are death. The banks of its refreshing streams, its rich and flowing meadows, the borders of its glassy lakes, the luxuriant plains

of its overflowing agriculture, the valley where the aromatic shrubs regale the eye and perfume the air, there are the chosen seats of this plague, the throne of Malaria. Death here walks hand in hand with the sources of life, sparing none: the labourer reaps his harvest but to die, or he wanders amid the luxuriance of vegetation and wealth, the ghost of man, a sufferer from his cradle to his impending grave; aged even in childhood, and laying down in misery that life which was but one disease. He is even driven from some of the richest portions of this fertile yet unhappy country; and the traveller contemplates at a distance deserts, but deserts of vegetable wealth, which man dares not approach, or he dies.

"Nor do even his homes and towns afford him a shelter against this all-pervading pestilence. It enters with him into his chamber, and stalks through his streets. Imperial Rome herself is its chosen victim: man flies before it, but the enemy is behind him and around him on all sides: every day sees the dominions of death extended, and the hour is impending when the Eternal City will cease to be, when it shall submit to that fate, which has been the fate of proud Niveveh, and Babylon the queen of nations.

"Such also is Sicily, such Sardinia, and such classic Greece."

If this vivid picture by Macculloch seems overdrawn, it should be recalled that as recently as 1930 not a single permanent household existed in all the Pontine Marsh area, one of the most fertile in Italy and within easy access of the huge markets of Rome (Hackett, 1937).

Malaria in Asia

Asia like Europe has been afflicted with malaria for centuries. India, for example, has suffered enormously. Sinton (1936), an expert malariologist, concluded that malaria by its direct and indirect effects was almost certainly responsible for at least two million deaths each year in India, including what is now Pakistan. Sinton further concluded that malaria was probably the greatest factor in lowering the health, vitality, and physical development of 100 million persons who were attacked each year, and that malaria caused financial losses of some 400 million dollars a year to the families affected, and incalculable losses to agriculture, industry, and commerce. Sinton concluded that the problem of existence itself in many parts of India was the problem of malaria which constituted the most important cause of economic misfortune, engendering poverty, diminishing the quality and quantity of the food supply, lowering the physical and educational standards of the nation, and hampering the increase of prosperity and economic progress in many ways.

India has been subject to epidemic as well as endemic malaria. In the Punjab, for example, time and time again malaria has demonstrated

a ferocity that staggers the imagination. For instance, in 1908 in the two months of October and November, fulminant malaria killed more than 300 thousand persons in this one province. In some towns the deaths exceeded 400 per 1000 population; the mortality rate in Amritsar, a city of 160,000, was over 200 per 1000 population. The entire community was prostrated and all ordinary business, including train service, was interrupted. For many weeks labor for any purpose was unprocurable and even food vendors ceased to carry on their trade. Following the thought of Christophers (1911) who studied the epidemic, it was as though a sea of miasm had rolled in upon the community, submerging it and suffocating an incredible number of the inhabitants.

Numerous other dramatic episodes could be recounted, for example, the terrible epidemic in Mauritius in 1867 when "the living were scarcely able to bury the dead"; in Brazil in 1938, when there were 100,000 cases with 14,000 deaths in a six-month period; and in Ceylon in 1934-1935 affecting a million and a half and killing nearly 70,000 persons in about six months. But far more important even than such cyclonic epidemics has been the insidious, anemia-producing, stunting effect of endemic malaria century after century throughout much of the tropics and subtropics.

Make no mistake about it. Malaria has had in the past tremendous power over masses of mankind, killing millions and blighting tens of millions each year. Moreover, so long as malaria persists in large seedbeds in the world it remains a potential threat. In the event of a serious breakdown in public services such as could occur in this atomic age, malaria might develop into epidemics that would compound disaster and greatly delay recovery from it.

So much for background. Now comes the pleasant task of discussing man's astounding and accelerating progress in mastering this mosquito-transmitted affliction. We have gone ahead so well that world-wide eradication of malaria seems possible in the foreseeable future.

Malaria Eradication

Malaria eradication is big news today! Seventy-six countries are planning, are carrying out, or have completed the eradication of malaria from their confines. Most authorities agree that eradication is preferable to conventional control because it sets a definite end-point, because it is cheaper in the long run, and because the growing phenomenon of *Anopheles* tolerance to residual insecticides indicates that the choice is either eradicating malaria or indefinitely enduring a disease transmitted by vectors that in increasing numbers are becoming resistant to control.

Malaria eradication implies both the complete interruption of trans-

mission and the elimination of the reservoir of parasites by a campaign limited in time and carried out so thoroughly that at its end there will be no resumption of transmission. But malaria eradication definitely does not imply the eradication of malaria mosquitoes, a goal rarely attainable and usually not economically possible.

Current malaria eradication programs have four phases: preparatory, attack, consolidation, and maintenance. The *phase of preparation* includes initial survey, planning, and preliminary operations. It generally lasts from several months to a year. The *phase of attack* begins as soon as the preparatory phase ends and continues with total spraying coverage until malaria transmission has ceased and the parasite reservoir has been nearly emptied. It has generally been found that most falciparum and vivax infections will have died out in 2½ to 3 years if there has been no reinfection. So three years is usually the minimum duration of an eradication attack phase. A few, not readily apparent, foci of residual malaria will generally remain at the end of the attack phase.

The *phase of consolidation* begins as the widespread spraying attack on the insect ends. During consolidation, residual pockets of transmission must be found and eradicated and the parasites remaining in man eliminated. Case-finding and the use of anti-malaria drugs have first importance. For this aspect of malaria eradication a new concept has arisen, that of *surveillance,* defined as that part of a malaria eradication project which is concerned with the discovery, the cure, and the prevention of such residual malaria infections in man as would delay the ending of transmission or threaten its resumption in a given area. Surveillance may be started in the attack phase but is most active in the phase of consolidation. This essential and rather difficult phase of consolidation ends when during three years of active surveillance no locally contracted infections have occurred. Because malaria mortality is so low during this key phase of an eradication project, it is sometimes difficult to obtain the necessary financial support from either governmental or international sources to carry the work through to completion. It is an important paradox in some areas that when malaria incidence becomes very low more money and attention than ever should be put into malaria eradication.

The final *phase of maintenance* begins when malaria has been eradicated from an area and lasts as long as malaria exists anywhere in the world. But maintenance is not difficult or expensive because it is carried out by regular health departments which add malaria to the list of exotic diseases against which they are always on guard.

In most large countries, time schedules will vary in different areas, some of the latter coming to the maintenance phase sooner than others.

World Status of Malaria Eradication

Accurate vital statistics are unobtainable in underdeveloped countries. Malaria in particular is subject to much confusion. Sometimes most fevers in an area are classified as malarial, sometimes most cases of malaria are not reported at all. But, due to the increased emphasis on malaria eradication during the past few years, with wider and more detailed surveys by better trained personnel, it is possible to present figures that probably are not misleading, although certainly they are not to be considered as more than carefully prepared estimates.

Very likely, about 45 per cent of the world's total population of 2,677 millions live in communities in which they are now or have very recently been exposed to malaria transmission. That is to say, when we talk about world-wide malaria eradication we mean the removal of all threat of malaria infection from some 1.2 billion people. That is quite a contract! How far along is it?

On the basis of data from WHO, it appears that by the end of 1957, country-wide malaria eradication projects were operating or nearing completion in countries having a total exposed population of some 248 million (total population, 576 million). Moreover, by the end of 1957, officially adopted malaria eradication schemes were being prepared in countries with a total exposed population of 480 million (total population 580 million). Thus in 1958, some 728 million of the 1.2 billion exposed to transmission will be under malaria eradication projects, about 61 per cent.

Expressed another way, there are in the world, according to the 1956 *UN Demographic Yearbook*, 196 nations, territories, dependencies, or administered areas of which 58 are non-malarious, leaving 138 to be considered in world-wide planning for malaria eradication. Seventy-six already are operating or preparing eradication projects; 62 still have no plans. Some of the latter, like Australia, Japan, and Singapore, have little malaria; others, like Pakistan, have a great deal. From one large country, Communist China, we have no information as to the current malaria situation. But world-wide malaria eradication requires that even faint endemicity and even Chinese malaria must be eliminated.

Countries now at or near malaria eradication are Argentina, British Guiana, Chile, France (Corsica), Cyprus, French Guiana, the Gaza strip, Italy (including Sardinia and Sicily), Martinique, Mauritius, Netherlands, Puerto Rico, Reunion, Romania, USA, and Venezuela.

A good example is Italy, the former malariousness of which I described earlier. The incidence of malaria in Italy in 1919 was over 300,000 cases and in 1945 it was over 400,000. In 1957, fewer than 100 cases were reported, mostly from a small overlooked Sicilian focus, now dealt with. Sardinia had not one case locally contracted and mosquito-borne.

I recall clearly my first visit to Sardinia in 1929, with Lewis Hackett. As we visited one poverty-stricken village after another, I became greatly depressed by the pitiable condition of the children, so many of whom were malarious. In the typical village street, almost always I would see sad little tots with huge bellies and pale faces, sitting listlessly in the doorways of their homes, seeming too weak to brush away the flies that ringed their eyes. My last visit was in 1950, at the close of the eradication project, and what a change I saw! The village children everywhere were playing vigorously, kicking balls and romping about in a normal healthy way, with rosy cheeks and no swollen bellies. Still some house flies but no longer able to linger about the eyes of the children. Sardinia is becoming a vacation resort and is rapidly erasing the evil name that malaria gave it from the time of the Caesars until some 10 years ago.

International Cooperation

Time will not permit me to go very far into the history of international cooperation in malaria control and eradication. But among the many facets of this subject, as it developed prior to the end of World War II, may be mentioned the Pan-American Sanitary Bureau, which has been concerned with malaria control since 1907; the second Pan-American Scientific Congress, held in Washington in 1916, which adopted a resolution calling on the American Republics to join in an effort to eradicate malarial diseases from the Western Hemisphere; the Malaria Commission of the Health Organization of the League of Nations, which functioned from 1923 until the onset of World War II; the United Nations Relief and Rehabilitation Administration, which from 1943 to 1946 contributed to malaria eradication, especially in Greece and Sardinia; the Institute of Inter-American Affairs, set up in 1942, which carried out extensive antimalaria works in Latin America; and finally, The Rockefeller Foundation, which between 1920 and 1954 supported cooperative programs in many overseas areas. It seems to me fair to state that The Rockefeller Foundation through its world-wide cooperative support of basic preparatory work in (1) epidemiology; (2) practical control demonstrations; (3) training programs; and (4) the establishment of official organizations and institutes for malaria study and control, had a major part in laying the foundations for the remarkable acceleration in man's attack on malaria that we are now witnessing.

Since the end of World War II, international cooperation in regard to malaria has blossomed magnificently. In fact, the world never before has had an international sanitary effort that approached in magnitude and effectiveness that now existing in the world-wide cooperative attack on malaria. With justification we sometimes view the world scene these days with pessimism, wondering if mankind has made any progress except in

gadgetry and military hardware. As Bertrand Russell (1957) said, the really important question today is not "will it be possible for man to inhabit other planets?", but "will it be possible for man to continue to inhabit his own planet?" This philosopher thinks that a favorable answer is possible only if we can learn to think in terms of the welfare of mankind and not in terms of the welfare of one's own nation or group. Social progress, however slow, has been made and it seems to me that the international aspect of the present world-wide drive against malaria is evidence of such progress.

Several bodies have sparked this international attack on malaria. First, great credit should go to the World Health Organization for its effective leadership in the amazing acceleration of world-wide malaria eradication since 1947. The Malaria Section, with the help of five WHO Regional Offices, has stimulated nation-wide projects, demonstrated the feasibility of residual spraying in many areas, provided fellowships and training courses, organized regional malaria conferences, fostered inter-country and inter-regional unanimity in regard to malaria eradication, and financed basic research.

WHO has set up a Malaria Eradication Special Account (MESA) to which have been invited contributions from governments, non-governmental organizations, and private sources. Monies from this account may be spent in direct aid to national eradication projects. The United States in December, 1957, made a gift of $5 million to this account, the first sizeable contribution it has had.

The Pan American Sanitary Organization with its own funds, and also as the Regional Office of WHO for the Americas, has had a key rôle in pushing forward the attack on malaria in the Americas. It is now coordinating and assisting a booming hemisphere-wide project that will drive malaria completely out of North and South America and the West Indies. The PASO also has a special malaria eradication fund to which the United States, Venezuela, and the Dominican Republic have made sizeable contributions. In December, 1957, the United States increased its support by putting an additional $2 million into the account.

The United Nations Children's Fund (UNICEF) has had a tremendous impact on malaria. Since 1947, this organization has put some $26.4 million into insecticides, equipment, and transport for malaria control and eradication in 56 countries. The 1958 malaria budget of UNICEF is $8 million. Here have been the sinews of war without which the impressive gains of the last few years would have been impossible.

The United States, as already implied, has also had a vital part in the world-wide attack on malaria. In addition to its relatively large share of the budgets of WHO, UNICEF, and PASO, an annual share totaling over $11 million, the United States through the International Cooperation Ad-

ministration (ICA) and its predecessors, has spent a total of $$8.9 million for malaria control and eradication in some 30 countries. The 1958 budget of ICA for malaria eradication in $23.3 million. The Division of International Health of the United States Public Health Service has had a major part in many policy decisions relating to malaria eradication and in providing personnel for ICA projects. This Division has been a powerful factor in recent world-wide victories over malaria.

Finally, it must be emphasized that very large sums have been and are being spent by national governments for malaria eradication within their borders. The monies that individual governments have provided for this purpose have equaled about 60 per cent of the total spent. Great credit must go to political leaders in those countries that have decided to budget national funds for the eradication of malaria.

Problems

Three resistance problems threaten to delay the accomplishment of world-wide malaria eradication. These are (1) resistance of anophelines to residual insecticides; (2) resistance of plasmodia to synthetic antimalarials; and (3) resistance of malaria in Central Africa to eradication.

Resistance to Insecticides

The problem of *Anopheles* resistance to residual insecticides is of prime interest to malariologists today. Among the fifty or more species that are significant vectors of malaria, behavioristic or physiologic resistance has been detected since 1951 in some part of the geographic range of seven, *viz. A. albimanus, gambiae, quadrimaculatus, sacharovi, stephensi, subpictus and sundaicus.* In one area, Greece, such resistance has clearly delayed a malaria eradication project.

This resistance phenomenon is not yet well understood but a brief account of some aspects of the subject may be of interest. To begin with, what we describe as a normal *Anopheles* mosquito will not habitually avoid contact with residual insecticides; if it does it is said to exhibit *behavioristic resistance.* Moreover, so-called normal anophelines (1) will freely absorb BHC, Dieldrin, or DDT; (2) will not detoxify or excrete these insecticides or store them as inactive deposits in tissue; and (3) do not have nerve axons or myoneural mechanisms that are unaffected by these toxicants. In other words, the so-called normal anopheline is susceptible to the residual insecticides and is killed by them. If the insect does not absorb the insecticide, or it can block or neutralize the usual lethal effects of such absorption it is said to exhibit *physiologic resistance.*

On the basis of studies by Macdonald (1957) and others it is apparent that physiologic resistance is hereditarily transmitted from parent *Anopheles* to offspring and that this inheritance follows the pattern associ-

ated with a single pair of genes, described as monofactorial. It also appears that specific physiologic resistance divides into at least two classes; in the first there is group resistance to BHC, Dieldrin, chlordane, and closely related compounds; in the second group the resistance is to DDT, methoxychlor, and near relatives. Within the group, resistance to one insecticide is accompanied by resistance to the others. But resistance to one group does not imply resistance to the other. So DDT is effective against Dieldrin-resistant *A. gambiae* in Northern Nigeria, and Dieldrin against DDT-resistant *A. sundaicus* in north Java.

Techniques are being developed to make it possible to determine on a practical scale in the field, in advance of a spraying program, what sort of physiologic resistance is present in a given species of a given area and, more than this, to find out the proportions of wholly resistant, hybrid, and wholly susceptible anophelines of a given species in a given area.

The problem of *Anopheles* resistance to insecticides is a real one although so far such resistance has not delayed the world-wide program seriously. But each year brings new reports of such resistance in hitherto unaffected areas. Obviously basic research is required to solve this annoying problem.

Drug Resistance

In over three centuries of widespread use no evidence of acquired parasite resistance to quinine has ever been demonstrated. Moreover, as regards the newer synthetic remedies, no acquired resistance has been demonstrated against plasmoquine, in use since 1925, quinacrine (Atabrine) in use since 1930, chloroquine (Aralen) in use since 1934, amodiaquine (Camoquine) in use since 1946, and primaquine, used since 1950.

But significant acquired resistance by plasmodia to chlorguanide (Paludrine), in use since 1945, and to pyrimethamine (Daraprim), used since 1951, has been reported from several areas. This acquired resistance is inheritable and quite stable, and it persists through repeated mosquito passages. The resistance has been manifested chiefly by schizonts but has also been observed in pre-erythrocytic forms and in gametocytes. Cross resistance between chlorguanide and pyrimethamine has also been demonstrated. While a genetic origin of this drug resistance is suggested, little is known about the actual mechanism of its development.

Chlorguanide promised great usefulness because of its low toxicity, low cost, high effectiveness as a prophylactic against falciparum malaria, and its sporontocidal action resulting in a marked inhibitory effect on malaria transmission by mosquitoes. So the appearance of resistance has posed a problem.

The other drug, pyrimethamine, is remarkable in its action (1) to effect complete suppression of quartan and suppressive cure of vivax and falci-

parum malaria in extremely small doses; and (2) to inhibit sporogony and thus to prevent malaria transmission (Covell *et al.*, 1955). The drug is tasteless and inexpensive, and has very low toxicity. So it is another drug of great promise that has become a resistance problem.

Resistance of Malaria in Central Africa

Malaria in Africa between the Tropics of Cancer and Capricorn has an extremely stable and relatively high endemicity based on several factors. For instance, the chief vector, *A. gambiae,* has a high man-biting frequency, Macdonald (1957) has estimated that the chance of this mosquito feeding on man during its lifetime is 136 times greater than the same chance in reference to *A. culcifacies* in Madras. *Anopheles gambiae* also has a relatively high expectation of life of 13 days as contrasted to 3.8 days of *culicifacies.* Furthermore, average temperatures in Central Africa are favorable to rapid completion of the development of the plasmodium in its mosquito host. The second of the principal vectors in Central Africa is *funestus,* not much inferior to *gambiae* in its vectorial powers.

Moreover, there are administrative difficulties due to the underdeveloped governmental services, communications, and especially public health departments. Yet the very high endemicity and the stability of malaria require greater and more sustained efficiency in eradication procedures than are usually needed elsewhere. Certain other difficulties, although not unique in Africa, are quite troublesome. For example, (1) the resistance of *gambiae* to Dieldrin in areas of West Africa; (2) the unfavorable reaction between wall surface and insecticide that curtails the effect of the toxicant, a phenomenon exhibited by many mud walls in Central Africa; and (3) outdoor feeding by *gambiae.*

On the other hand, a possible advantage in Central Africa is the preponderance of falciparum malaria over vivax. We possess against falciparum the drug chloroquine, which produces a radical cure and which can be administered to masses of individuals with a minimum of medical supervision. However, we need more suitable formulations for mass use of this drug.

So, Central Africa presents problems for solution. Several studies are going forward and preliminary reports of results in Northern Nigeria are good. But obviously much more study must go into the problem of malaria eradication in Central Africa.

Conclusions

World malaria incidence has fallen by tens of millions in the past few years under the impact of modern residual insecticides applied on a nation-wide scale in many countries, with the leadership, technical, and

financial aid of WHO, PASO, UNICEF, ICA, and progressive national health departments. Advances have been so great that world-wide malaria eradication seems a practical goal although we still have a long road to travel. As previously stated, there were probably 200 million cases of malaria throughout the world in 1957 with two million malaria deaths. There is need for much more international monetary and professional assistance, for more trained malariologists, and particularly for more malaria research.

Undoubtedly, the difficulties of world-wide malaria eradication are enormous, but so are the potential resources of money and technological skills that can be put to the task if there is the will to do so. We face a golden moment that intelligently utilized will lead us to a tremendous international public health victory.

REFERENCES

Ackerknecht, E. H. 1945. *Malaria in the Upper Mississippi Valley,* 1760-1900. The Johns Hopkins Press, Baltimore. 142 pp.

Barber, M. A. 1929. The history of malaria in the United States. *U.S. Publ. Health Rep'ts,* **44,** 2575-2587.

Christophers, S. R. 1911. Malaria in the Punjab. *Scientific Memoir, Gov't of India,* 46, Delhi. 135 pp.

Covell, Sir Gordon, Coatney, G. R., Field, J. W., and Singh, J. 1955. *Chemotherapy of Malaria.* World Health Org. Geneva. 123 pp.

Duffy, John. 1953. *Epidemics in Colonial America.* Louisiana Univ. Press, Baton Rouge.

Dunn, F. L. 1957. Personal communication from Communicable Disease Center, *U.S. Publ. Health Serv.,* Atlanta, Ga.

Gregg, Alan. 1956. *Challenges to Contemporary Medicine.* Columbia Univ. Press, New York. p. 28.

Hackett, L. W. 1937. *Malaria in Europe.* Oxford University Press, London. pp. 316-317.

Hackett, L. W. 1949. Conspectus of malaria incidence in Northern Europe, the Mediterranean region, and the Near East. In Boyd's *Malariology.* pp. 788-799. W. B. Saunders Company, Philadelphia.

Macculloch, John. 1829. *Malaria; an Essay on the Production and Propagation of this Poison, etc.* pp. 7-8, p. 190. Thomas Kite, Philadelphia.

Macdonald, George. 1957. *The Epidemiology and Control of Malaria.* Oxford Univ. Press, London. 201 pp.

Petersen, W. J. 1938. Doctors, drugs and dentists. *Palimpsest.* **19,** 401-412.

Russell, Bertrand. 1957. Can scientific man survive? *The Saturday Review.* Dec. 21. pp. 24-25.

Russell, Paul F. 1955. *Man's Mastery of Malaria.* Oxford Univ. Press, London. 308 pp.

Russell, Paul F. 1956. World-wide malaria distribution, prevalence, and control. *Am. J. Trop. Med. Hyg.* 5, 937-965.

Sinton, J. A. 1936. What malaria costs India, nationally, socially, and economically. *Records Malaria Survey of India.* 6, 91-169.

Williams, L. L., Jr. 1938. Economic importance of malaria control. *New Jersey Mosq. Extermination Assoc. 25th Ann. Meeting.* New Brunswick. pp. 148-151.

THE ENIGMA OF HOST-PARASITE RELATIONS IN AMEBIASIS

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"The clear definition of a difficulty frequently hastens the advance of science by indicating a pitfall or a wrong road of inquiry."
CLIFFORD DOBELL. 1919.

IN A GENERAL way it may be said that the host-parasite relationship in any infection is concerned with the interaction between parasite and host, which are in constant conflict, the virulence of the former struggling to overcome the resistance of the latter. In the case of amebiasis, the progress in our knowledge of the actions and reactions of the two components of the host-parasite system has been unequal, and certain aspects of their association are still obscure.

The successful maintenance of *Entamoeba histolytica* in cultures and recent developments in the technique of animal experimentation have provided useful tools for the study of this problem, but, while notable advances have been made in our knowledge concerning the life history of the parasite and its effect upon the host, very little is known about the way the host reacts against the amebic infection. And indeed, most of the information regarding immunity and resistance in amebiasis is based upon circumstantial evidence rather than upon experimentally established facts.

ACTION OF PARASITE ON HOST

Unicism. For a critical appraisal of the role played by the human host in the course of amebiasis, it will be necessary to review the available data regarding the behavior of *E. histolytica* in the infection. One of the most important facts concerning the etiology of amebiasis is that, in the great majority of cases of infection throughout the world, *E. histolytica* lives in the lumen of the human gut as a commensal without causing any damage to its wall or detectable symptoms of disease (Hoare, 1952). Furthermore, among these symptomless infections a distinction should be made between those with the small and large races of *E. histolytica.* It is now generally admitted that the small race is inoffensive and—as far as its medical importance is concerned—should therefore be placed among the other non-pathogenic amebae of man, such as *E. coli, Endolimax* and *Iodamoeba* (Hoare, 1952, 1957; Ridley & Schofield, 1957). Moreover, the

small race is sufficiently characterized both morphologically and biologically to be regarded as a distinct species or subspecies, under the name *E. hartmanni* or *E. histolytica hartmanni*, respectively (Burrows, 1957; Hoare, 1949, 1952, 1957).

The exclusion of *E. hartmanni* leaves only the large race (*E. histolytica* proper) for consideration as a pathogen. In the past various hypotheses have been proposed to explain variations in the course of amebic infections. Thus, some authors maintain that, although in many cases the large race of *E. histolytica* lives in the lumen of the gut as a commensal ("minuta" form), feeding there on bacteria and saprozoically, it is potentially always pathogenic but its virulence (or invasiveness) remains dormant until activated by extraneous factors (such as harmful bacteria, functional disturbances, host's diet, which will be considered below) which affect the host's health, thereby lowering his resistance and impairing the structural integrity of the intestinal wall.

However, in discussing the possible factors affecting the pathogenicity of *E. histolytica* the characteristics of different strains have usually not been taken into consideration, though it is a well-known fact that the course of amebiasis varies considerably in different parts of the world. Thus, although the incidence of infection in countries with a temperate climate is comparable to that in hot countries, manifestations of disease (including dysentery) are common in the tropics but rare in temperate regions, where the infections are as a rule symptomless. Until quite recently it was generally assumed that *E. histolytica* is represented by one race of pathogenic amebae and that variations in the course of amebiasis observed under different climatic conditions were determined by various injurious factors mentioned above, which in the tropics tend to lower the host's resistance to invasion by the amebae. It is true that some observers suggested that the types of amebiasis prevalent in regions with different climates might be due to the existence of virulent strains in the tropics and of avirulent ones in temperate regions. However, it was thought that the invasive power was not an innate peculiarity of the amebae but depended on the conditions previously experienced by them in the host. Thus it was suggested that the virulence of a strain might be enhanced as the result of adaptation to life in the tissues (Reichenow, 1931), a view supported by experiments in which the virulence of *E. histolytica* was apparently increased by repeated passages through animals (Faust & Swartzwelder, 1935; Meleney & Frye, 1937). And, conversely, it was believed that prolonged sojourn in the lumen of the gut might reduce the invasiveness of this ameba (Westphal, 1950).

Dualism. This unicistic concept was opposed by Brumpt (1925, 1928, 1949) who propounded a dualistic hypothesis of the etiology of amebiasis.

According to his view, the large race of amebae with quadrinucleate cysts comprises two types, which he regarded as independent species, *E. dispar* and *E. dysenteriae* (= our *E. histolytica*). While *E. dispar* is a nonpathogenic ameba inhabiting the human intestine throughout the world, *E. dysenteriae* is the only pathogenic form responsible for amebic dysentery and other clinical manifestations in warm and hot countries to which this parasite is confined, though its virulence may be in abeyance in carriers. Brumpt thus believed that the only ameba indigenous to countries with a temperate climate was the harmless *E. dispar*, while the pathogenic *E. dysenteriae* is only occasionally introduced there by persons who contracted their infection in hot countries. Among the arguments in favor of the independence of these two species Brumpt (1928) pointed out that none of the factors which promote the development of clinical symptoms in infections with *E. dysenteriae* has any effect on carriers of *E. dispar* in temperate climates: thus, despite the presence of typhoid fever and bacillary dysentery in France, amebic dysentery does not occur there. On the other hand, in convalescent carriers, who had settled in France, the exotic *E. dysenteriae* does not lose its virulence. In other words, *E. dispar* and *E. dysenteriae* "breed true."

Strain variation. Brumpt's ideas, which were based on epidemiological data, were received with some scepticism during his life, but they have now been vindicated by experimental investigations on the virulence of strains of *E. histolytica* isolated from patients with different types of amebiasis. Although recent work on these lines fully supports Brumpt's main thesis, the conclusions derived from earlier experiments were contradictory (Dale & Dobell, 1917; Kessel, 1928; Meleney & Frye, 1933, 1935, 1937 and others), owing to misleading results obtained with kittens, whose susceptibility and vulnerability to infection with *E. histolytica* are exceptionally high. But with the introduction of rodents as experimental hosts this question could be studied under conditions more closely resembling those found in the human host. During the last few years this subject has been thoroughly investigated by Neal (1951a, b; 1954, 1956a; 1957; Neal & Vincent, 1955, 1956), who demonstrated that strains from symptomless human infections were invariably avirulent, producing no lesions in experimentally infected rats, whereas strains from clinical cases invaded the gut wall, causing typical ulceration. It was also shown that the invasiveness of these strains was a stable property which could not be modified by interchange of their floras, by diet, or by animal passages, for through all these manipulations the strains retained their original peculiarities. The avirulence of strains from symptomless cases and the virulence of strains from clinical cases were recently confirmed in rabbits by Hunninen & Boone (1957), while Beaver *et al.* (1956), who infected 46

volunteers with a carrier strain, found that it produced in all of them symptomless infections persisting in some cases up to 14 months.

Neo-dualism. Since the invasiveness of a strain cannot be altered experimentally, its characteristics appear to be fixed, indicating the existence of two types of *E. histolytica*: (1) the non-virulent one, corresponding to Brumpt's *E. dispar*, and (2) the virulent one, corresponding to his *E. dysenteriae*. In the light of these observations our concept of the host-parasite relations and etiology of amebiasis takes on a "new look": though corresponding to Brumpt's original views, it leaves the question regarding the validity of the specific independence of the two races open. This neo-dualistic hypothesis thus postulates that the human entamebae with 4-nucleate cysts are represented by the following categories: the small non-pathogenic *E. hartmanni* and the large *E. histolytica*, which in its turn comprises two types or races that are morphologically identical but differ in virulence, one being a non-pathogenic commensal, the other a pathogen capable of invading the host's tissues with the production of the well-known clinical manifestations of amebiasis. The invasive large race is restricted to warm and hot countries, where clinical amebiasis is endemic, whereas *E. hartmanni* and the avirulent large race of *E. histolytica* are apparently cosmopolitan and are the only indigenous parasites in countries with a temperate climate, though clinical cases of exotic origin are occasionally introduced there. In this connection it is significant that in a number of European countries (e.g. Britain, Germany, Holland, France) there is either direct proof or strong circumstantial evidence that local cases of amebic dysentery are due to importation of virulent strains of *E. histolytica* or have been in contact with extraneous sources of infection (cf. Hoare, 1950; Boyd, 1957).

It has been estimated (cf. Hoare, 1952) that the average prevalence of infection with *E. histolytica (sensu lato)* throughout the world is about 20% of the population, representing a global incidence of more than 400 million cases. Of this number roughly 80% have no signs or symptoms of disease, about one-third of them harboring the inoffensive small race *(E. hartmanni)* and the rest being infected with the large races. Among the symptomless infections with the large amebae, a high proportion are cases of non-pathogenic amebiasis harboring the avirulent race, whereas those parasitized with the virulent race are carriers in whom—under favorable conditions—clinical manifestations are liable to develop at any time. If this interpretation of the host-parasite relations in amebiasis is correct, the only parasite concerned with the etiology of the clinical disease is the virulent race of *E. histolytica*, but, at the present stage of our knowledge, in cases without clinical manifestations it can be differentiated from the avirulent race only by animal experimentation.

The changing views on the etiology of amebiasis are summarized in the following table:

ETIOLOGY OF AMEBIASIS

HYPOTHESES	UNICISTIC 1913–	DUALISTIC 1925–	NEO-DUALISTIC 1957
	E. histolytica		
NON-PATHOGENIC	SMALL RACE	*E. hartmanni*	*E. hartmanni*
AMEBIASIS	LARGE RACE:		
	Commensal phase ("minuta" form)	*E. dispar*	*E. histolytica* Avirulent large race
PATHOGENIC			*E. histolytica* Virulent large race:
AMEBIASIS		*E. dysenteriae*	
Subclinical			Dormant in lumen of gut
	↑		↑
	Virulent (tissue) phase ("magna" form)		↓
Clinical			Invading gut wall

Pathogenesis. We now turn to a consideration of the factors which are held to be responsible for the activation of the dormant virulence of the invasive race of *E. histolytica.* One of the most important factors influencing the course of amebic infection is the intestinal flora. It is well known that the role played by bacteria in the life of *E. histolytica* is so essential that their association appears to be almost symbiotic. Thus, there is conclusive evidence that bacteria (or other suitable *living* cells) are indispensable for the maintenance and development of this ameba *in vitro* (cf. Dobell & Neal, 1952), and it has been demonstrated *in vivo* that the amebae are unable to establish an infection in "germ-free" animals (Phillips *et al.*, 1955). The intestinal flora not only serves as a source of food and of some unknown growth factors promoting the survival of *E. histolytica* in the lumen of the host's gut, but, according to many authors (Westphal, 1937, 1938; Deschiens, 1938; Phillips *et al.*, 1955, and others), it plays a major part in the pathogenesis of amebiasis, especially when virulent bacteria are present (Westphal, 1937, 1948). According to this view, the normal mucosa of the gut wall is resistant to penetration by the

amebae, but, when it is injured by virulent bacteria and their toxins, the way is paved for the amebae, which then invade the tissues and produce the characteristic lesions. On the other hand, there is some evidence at present that the amebae take a more active part in this process, and some authors even believe that penetration of the tissues can be effected by their own unaided efforts (Meleney *et al.*, 1939; Phillips & Bartgis, 1954; Neal, 1957). Nevertheless, the available facts strongly support the view that bacteria play an important contributory role in the production of lesions in amebiasis (Porter, 1953; Phillips *et al.*, 1955).

In addition to bacteria, it has been suggested that other causes of disturbed gastro-intestinal function may expose the intestine to invasion by *E. histolytica*, such as abnormal secretory function, temperature fluctuations and irritant foodstuffs (Westphal, 1938), as well as inflammatory and erosive processes manifested by colitis (Deschiens, 1950a). Some of these factors are held to be responsible for the prevalence of clinical amebic dysentery in the tropics, where inadequacies of diet and bacterial assault commonly provoke gastro-intestinal disorders and a lowering of the host's resistance. It has also been shown that variation in the host's diet and its vitamin content may be predisposing factors in amebiasis (this question has recently been fully reviewed by Porter, 1953; Frye, 1955; and Chandler, 1955, 1957). These are the main conditions which are thought to influence the course of amebic infection, but the data are inconclusive and it is obvious that the question of pathogenesis in this disease is in need of further investigation.

A good illustration of the potential pathogenicity of carrier strains from endemic areas of clinical amebiasis is provided by observations made during the First World War. On the one hand, it was shown (Woodcock, 1917) that 20% of the normal population of India were symptomless carriers of *E. histolytica*. On the other hand, it was found that both in Egypt (Woodcock, 1917) and in Mesopotamia (Ledingham, 1920) cases of amebic dysentery were far more frequent among the Indian troops than among British troops operating in these countries. In the light of our present knowledge of the etiology of amebiasis, it is conceivable that the Indians already harboured pathogenic strains, the dormant virulence of which was activated when amebic carriers were exposed to unfavorable conditions on the front. As regards the low incidence of disease among the British, it was probably due to the fact that local invasive strains had not yet succeeded in infecting many of them.

Attenuation of virulence. One of the puzzling phenomena in amebiasis is the failure of virulent exotic strains to establish themselves in temperate regions. Thus, after the First and Second World Wars, when troops which had been exposed to infection with virulent strains of *E. histolytica* in

opical countries were returning to Europe and North America, medical thorities were much concerned about the possibility of the introduc- on and spread of clinical amebiasis among the local inhabitants: yet othing happened. It is possible that convalescent carriers of exotic rains are present among us, but no relapses are occurring owing to the sence of predisposing factors. On the other hand, it is known (De- hiens, 1939, 1941; Chang, 1945; Neal, 1956a) that a virulent strain may come attenuated after prolonged cultivation, though its virulence can e restored by animal passages (Neal & Vincent, 1956). Since in a temper- e climate the amebae usually live in the lumen of the gut in association ith the conventional flora, it is conceivable that exotic strains of *E. his- lytica* may gradually lose their virulence—as they do *in vitro*—and this ight account for the scarcity of clinical cases in countries with a colder imate.

Invasion. There has been much speculation about the mechanism of ssue invasion by *E. histolytica.* The histological picture of early lesions dicates that the parasite elaborates a substance causing histolysis of the ssues, and various views have been advanced regarding its nature. While me authors (Rees, 1929; Meleney, 1944; Frye & Shaffer, 1948) thought at the secretion was a toxin, others found no signs of toxic effects (e.g. crosis) in the lesions, nor were they able to reproduce symptoms of in- xication in rabbits inoculated with extracts of amebic cultures (West- al, 1938). Those who held that penetration of the tissues by the amebae, d their pathological effect, are due to fermentative action sought to entify the enzymes responsible. Thus, over 30 years ago Craig (1927) scovered in *E. histolytica* a cytolytic substance ("cytolysin"); later Rees *al.* (1953) demonstrated production of a gelatinase, and Deschiens 950b) noted its ability to hydrolyse proteins *in vitro.* Recently Bradin 953) discovered the presence of hyaluronidase in this ameba and sug- sted that it promotes tissue invasion, but DeLamater *et al.* (1954) failed detect this enzyme. Finally, Neal (1956b) demonstrated the presence of oteolytic enzymes in this ameba.

It is thus seen that the evidence regarding the nature of the enzymes sponsible for the invasion of the tissues by *E. histolytica* is still frag- entary and inconclusive. However most observers are agreed that this neba produces some proteolytic enzyme, which liquefies the intestinal ucosa at the point of application, thereby enabling it to penetrate into e tissues, which continue to break down by histolysis as the amebae ltiply and extend through the ulcers. In addition to the fermentative ocess, there can be no doubt that the progress of the amebae within the sues is aided in a purely mechanical manner by their locomotion (Hoare Neal, 1955).

Reaction of Host to Parasite

Having reviewed the present state of knowledge regarding the behavior of the parasite we are in a better position to discuss the question of immunity in amebiasis. Among the available facts the first in order are the well-known classical experiments of Walker & Sellards (1913), who had shown that human beings varied in their susceptibility to infection with *E. histolytica*, one and the same strain of which produced symptomless infection in some individuals and clinical manifestations in others. These results were interpreted as evidence for the existence of natural or innate immunity in certain persons (Dobell & Low, 1922; Craig, 1944; Craig & Faust, 1951; Frye, 1955). Similarly, the absence of clinical symptoms in carriers has also been attributed to natural resistance of the host (Craig, 1944). On the other hand, there is no clear evidence of acquired immunity to amebiasis in man, for persons who had recovered from or were cured of an infection are liable to be reinfected (Craig, 1944; Anderson *et al.*, 1953; Porter, 1953; Frye, 1955), and it is significant in this connection that amebic infections are remarkably persistent, lasting sometimes for years (Dobell & Low, 1922; Brumpt, 1928, 1949). That the human host does not acquire immunity to reinfection has recently been demonstrated experimentally (Beaver *et al.*, 1956), though dogs which had recovered from the disease appear to be resistant (Simić, 1935; Swartzwelder & Avant, 1952).

Among the barriers which an intestinal parasite like *E. histolytica* must overcome to gain access to the tissues is the normal mucosa of the gut wall. When its resistance is lowered by various factors mentioned above this barrier may be broken at some points, exposing the tissues to attack by the amebae (Westphal, 1937, 1938; Fischer & Reichenow, 1952; Anderson *et al.*, 1953; Chandler, 1957). However, as noted already, it is not clear whether or not the amebae are capable of penetrating through this barrier without the aid of extraneous agents.

More tangible—though indirect—indications of some immunological response to amebic infection is provided by serological reactions, the most important of which is the complement fixation test. Though much work has been devoted to this test, the results are conflicting, some of the discrepancies probably being due to variations in the preparation of antigens and in technical procedure, or to antigenic differences between strains of *E. histolytica* used for the test (Hussey & Brown, 1950; Bozicevich, 1950). However, there seems to be a consensus that when the amebae are restricted to the lumen of the gut (as in symptomless infections) they do not stimulate antibody formation and the reaction is therefore negative, but when the parasite invades the tissues there is a positive

erological response, which is weak in the case of intestinal involvement nd stronger in the case of amebic hepatitis (Hussey & Brown, 1950; leinz *et al.*, 1956; Meleney, 1957).

Among other reactions for the demonstration of immune bodies in mebiasis, should be mentioned an intradermal test (Leal, 1953), and sts in which the presence of antibody in immune serum is revealed by nmobilization of the amebae (Cole & Kent, 1953; Brown & Whitby, 1955; alentino, 1956) or by their fluorescence when combined with fluorescein-gged antibody (Goldman, 1953).

If the foregoing data on immunity in amebiasis are examined in the ght of our present knowledge of the host-parasite relations, most of them ill not stand the test of critical analysis. And, indeed, individual variation susceptibility to infection with virulent strains of *E. histolytica* is prob-bly due mainly to diversity in the intestinal flora, rather than to disparity the innate immunity of different persons (as already suggested by auss & Rappaport, 1940, and Porter, 1953). It would seem, however, that endemic areas the indigenous population might possess a degree of acial immunity. Thus, according to Boyd (1957), in 1930-1935 the in-idence of amebic dysentery among British troops serving in India was igher than among Indian troops, in spite of the prevalence in that coun-y of pathogenic strains of *E. histolytica.* Likewise, the absence of symp-oms may be due either to infection with the avirulent race or to a lack f factors capable of activating the dormant virulent race. There is also airly convincing evidence that the human host does not acquire immu-ity in the course of his infection. There remain the serological reactions, hich do seem to point to some immunological response to amebic in-ection. Furthermore, the absence of inflammatory reactions to invasion y the amebae also indicates that the mechanism of defense is repre-ented by humoral rather than cellular factors (Anderson *et al.*, 1953).

Since no complement-fixing rection is produced in infections restricted the lumen of the gut, it would seem that the great majority of cases of mebiasis develop no specific immunity against *E. histolytica.* As regards ases in which invasion of the tissues has taken place, only a slight im-une response is detectable in them by serological reactions. From these cts it can be inferred that the amebic antigen is too weak to stimulate ppreciable antibody formation in the human organism. In this respect . *histolytica* is like many other pathogenic protozoa, the antigenicity of hich is of a low grade, producing only transient immunity. The anti-enic lability of protozoa has been attributed to the predominance in eir body of lipid haptens, which are unable to induce lasting immunity, contrast to carbohydrate haptens in bacteria, which provide powerful

antigens with a high antibody response (Kligler *et al.*, 1936, 1940). In th connection it may be significant that Ray & Sen Gupta (1954) have actuall detected the presence of lipids in the cytoplasm of *E. histolytica.*

Conclusion

The views advanced by me in this survey may appear to be somewha unorthodox, but they only represent deductions which are consistent wit the mass of observational and experimental data on amebiasis that hav accumulated up to date. Though a coherent pattern is emerging, the pi ture is by no means clear-cut, for many details are but faintly outline and much work remains to be done before the enigma of host-parasit relations in amebiasis is fully solved.

REFERENCES

Anderson, H. H., Bostick, W. L. & Johnstone, H. G. 1953. *Amebiasis: pathology diagnosis and chemotherapy.* Springfield, Ill.

Beaver, P. C., Jung, R. C., Read, T. R. & Robinson, T. A. 1956. Experiment *Entamoeba histolytica* infections in man. *Am. J. Trop. Med. Hyg.* **5**, 1000.

Boyd, J. S. K. 1957. Dysentery: some personal experiences and observations. *Tran R. Soc. Trop. Med. Hyg.* **51**, 471.

Bozicevich, J. 1950. [Discussion of paper by Hussey and Brown (*q.v.*)]. Am. J. Tro *Med.* **30**, 154.

Bradin, J. L. 1953. Studies on the production of hyaluronidase by *Endamoeba hist lytica. Exper. Parasitol.* **2**, 230.

Brown, J. A. H. & Whitby, J. L. 1955. An immobilization test for amoebiasis. *J. Cli Path.* **8**, 245.

Brumpt, E. 1925. Étude sommaire de l'*Entamoeba dispar*, n.sp., amibe à kyst quadrinuclées, parasite de l'homme. *Bull. Acad. Méd.* **94**, 943.

Brumpt, E. 1928. Differentiation of the human intestinal amoebae with four-nucleate cysts. *Trans. R. Soc. Trop. Med. Hyg.* **22**, 101.

Brumpt, E. 1949. *Précis de parasitologie*, 6th ed., Paris.

Burrows, R. B. 1957. *Endamoeba hartmanni. Am. J. Hyg.* **65**, 172.

Chandler, A. C. 1955. *Introduction to parasitology*, 9th ed. New York.

Chandler, A. C. 1957. Interrelations between nutrition and infectious disease in th tropics. (Presidential address). *Am. J. Trop. Med. Hyg.* **6**, 195.

Chang, S. L. 1945. Studies on *Entamoeba histolytica.* V. On the decrease in infectivi and pathogenicity for kittens of *E. histolytica* during prolonged cultivation an restoration of these characters following encystment and direct animal passag *J. Inf. Dis.* **76**, 126.

Cole, B. A. & Kent, J. F. 1953. Immobilization of *Endamoeba histolytica in vitro* b antiserum produced in the rabbit. *Proc. Soc. Exper. Biol.* **83**, 811.

Craig, C. F. 1927. Observations upon the hemolytic, cytolytic and complemen binding properties of extracts of *Endamoeba histolytica. Am. J. Trop. Med.* **7**, 22

Craig, C. F. 1944. *The etiology, diagnosis and treatment of amebiasis.* Baltimore.

Craig, C. F. & Faust, E. C. 1951. *Clinical parasitology*, 5th ed. London

Dale, H. H. & Dobell, C. 1917. Experiments on the therapeutics of amebic dysenter *J. Pharmacol.* **10**, 399.

DeLamater, J. N., Michaelson, J. B., Hallman, F. A. & Blumenthal, H. 1954. A investigation into hyaluronidase as a factor in the mechanism of tissue invasion b *Endamoeba histolytica. Am. J. Trop. Med. Hyg.* **3**, 1.

Deschiens, R. 1938. Le rôle de la flore bactérienne, associée à l'amibe dysentériqu dans l'amibiase. *Ann. Inst Pasteur*, **61**, 5.

)eschiens, R. 1939. Le pouvoir pathogène des amibes dysentériques en culture, ses relations avec l'enkystement. *Bull. Soc. Path. Exot.* **32**, 923.
)eschiens, R. 1941. Nouvelles données sur la relation existant entre l'enkystement et la conservation du pouvoir pathogène des amibes dysentériques, en culture. *Ann. Inst. Pasteur.* **67**, 468.
)eschiens, R. 1950a. La biologie de l'amibe dysentérique dans ses relations avec l'amibiase. *Gaz. Méd. France.* **57** (16), 829.
)eschiens, R. 1950b. La nutrition de l'amibe dysentérique. *Biol. Méd.* **39**, 57.
)obell, C. & Low, G. C. 1922. Amoebiasis. In Byam & Archibald's *Practice of Medicine in the Tropics*, v. 2. London.
)obell, C. & Neal, R. A. 1952. Researches on the intestinal protozoa of monkeys and man. XII. Bacterial factors influencing the life history of *Entamoeba histolytica* in cultures. *Parasitology.* **42**, 16.
aust, E. C. & Swartzwelder, J. C. 1935. Effect of continuous passage of *Endamoeba histolytica* through experimental dogs. *Proc. Soc. Exper. Biol.* **32**, 954.
ischer, L. & Reichenow, E. 1952. Amoebiasis. In *Handbuch der inneren Medizin.* **1** (2), 616.
rye, W. W. 1955. Nutrition and intestinal parasitism. *Ann. N.Y. Acad. Sci.* **63**, 175.
rye, W. W. & Shaffer, J. G. 1948. Experimental pathology of amebiasis. *Proc. 4th Int. Congr. Trop. Med. Malar.* Washington. **2**, 1075.
oldman, M. 1953. Cytochemical differentiation of *Endamoeba histolytica* and *Endamoeba coli* by means of fluorescent antibody. *Am. J. Hyg.* **58**, 319.
einz, H. J., Brauns, W. & MacNab, G. M. 1956. A new antigen for the amoebic complement fixation test. Interim report. *S. Afric. J. Med. Sci.* **21**, 9.
oare, C. A. 1949. *Handbook of medical protozoology.* London.
oare, C. A. 1950. Amoebiasis in Great Britain, with special reference to carriers. *Brit. Med. J.* **2**, 238.
oare, C. A. 1952. The commensal phase of *Entamoeba histolytica. Exper. Parasitol.* **1**, 411.
oare, C. A. 1957. Symposium on the laboratory aspects of amoebiasis. I. Introduction. *Trans. R. Soc. Trop. Med. Hyg.* **51**, 303.
oare, C. A. & Neal, R. A. 1955. Host-parasite relations and pathogenesis in infections with *Entamoeba histolytica.* In *Mechanisms of Microbial Pathogenicity* (5th Symposium of Soc. Gen. Microbiol.). London: p. 230.
unninen, A. B. & Boone, H. A. 1957. Studies on the pathogenicity of various strains of *Entamoeba histolytica* in the rabbit. *Am. J. Trop. Med. Hyg.* **6**, 32.
ussey, K. L. & Brown, H. W. 1950. The complement fixation test for hepatic amebiasis. *Am. J. Trop. Med.* **30**, 147.
essel, J. F. 1928. Amoebiasis in kittens infected with amoebae from acute and "carrier" human cases and with tetranucleate amoebae of the monkey and of the pig. *Am. J. Hyg.* **8**, 311.
ligler, I. J. & Olitzki, L. 1936. The antigenic composition of *Trypanosoma evansi. Ann. Trop. Med. Parasit.* **30**, 287.
ligler, I. J., Olitzki, L. & Kligler, H. 1940. The antigenic composition and immunizing properties of trypanosomes. *J. Immunol.* **38**, 317.
eal, R. A. 1953. *Intradermo-reaçcão na amebiase: contribuição para o seu estudo.* (Thesis: São Paulo).
edingham, J. C. G. 1920. Dysentery and enteric disease in Mesopotamia from the laboratory standpoint. *J. R. Army Med. Corps.* **34**, 189, 306.
eleney, H. E. 1944. The relationship of clinical amoebiasis to various strains and growth requirements of *Endamoeba histolytica. Puerto Rico J. Publ. Hlth. Trop. Med.* **20**, 59.
eleney, H. E. 1957. Some unsolved problems in amebiasis. *Am. J. Trop. Med. Hyg.* **6**, 487.
eleney, H. E. & Frye, W. W. 1933. Studies of *Endamoeba histolytica* and other

intestinal protozoa in Tennessee. V. A comparison of five strains of *E. histolytic* with reference to their pathogenicity for kittens. *Am. J. Hyg.* **17**, 637.

Meleney, H. E. & Frye, W. W. 1935. Studies of *Endamoeba histolytica* and othe intestinal protozoa in Tennessee. IX. Further observations on the pathogenicity certain strains of *E. histolytica* for kittens. *Am. J. Hyg.* **21**, 422.

Meleney, H. E. & Frye, W. W. 1937. The pathogenicity of four strains of *Endamoeb histolytica* from Chicago. *Am. J. Dig. Dis.* **4**, 37.

Meleney, H. E., Frye, W. W. & Leathers, W. S. 1939. The effect of prolonge cultivation on the pathogenicity of various strains of *Endamoeba histolytica* f kittens. *Am. J. Hyg.* **29C**, 61.

Nauss, R. W. & Rappaport, I. 1940. Studies on amebiasis. I. Pathogenesis of mucos penetration. *Am. J. Trop. Med.* **20**, 107.

Neal, R. A. 1951a. Some observations on the variation of virulence and response chemotherapy of strains of *Entamoeba histolytica* in rats. *Trans. R. Soc. Trop. Me Hyg.* **44**, 439.

Neal, R. A. 1951b. The duration and epidemiological significance of *Entamoeb histolytica* infections in rats. *Trans. R. Soc. Trop. Med. Hyg.* **45**, 363.

Neal, R. A. 1954. The influence of encystation upon the virulence of *Entamoeb histolytica* in rats. *Trans. R. Soc. Trop. Med. Hyg.* **48**, 533.

Neal, R. A. 1956a. Strain variation in *Entamoeba histolytica*. III. The influence of th bacterial flora on virulence to rats. *Parasitology.* **46**, 183.

Neal, R. A. 1956b. Proteolytic enzymes in *Entamoeba histolytica*. *Nature,* **178**, 59

Neal, R. A. 1957. Symposium on the laboratory aspects of amoebiasis. III. Virulen in *Entamoeba histolytica*. *Trans. R. Soc. Trop. Med. Hyg.* **51**, 313.

Neal, R. A. & Vincent, P. 1955. Strain variation in *Entamoeba histolytica*. I. Correl tion of invasiveness in rats with clinical history and treatment of the experiment infections. *Pasasitology.* **45**, 152.

Neal, R. A. & Vincent, P. 1956. Strain variation in *Entamoeba histolytica*. II. Th effect of serial liver passage on the virulence. *Parasitology.* **46**, 173.

Phillips, B. P. & Bartgis, I. L. 1954. Effects of growth *in vitro* with selected microbi associates and of encystation and excystation, on the virulence of *Endamoeb histolytica* for guinea pigs. *Am. J. Trop. Med. Hyg.* **3**, 621.

Phillips, B. P., Wolfe, P. A., Rees, C. W., Gordon, H. A., Wright, W. H. Reyniers, J. A. 1955. Studies on the ameba-bacteria relationship in amebiasis. Com parative results of the intracaecal inoculation of germfree mono-contaminated, ar conventional guinea pigs with *Entamoeba histolytica*. *Am. J. Trop. Med. Hyg.* 675.

Porter, R. J. 1953. Amebiasis. *Ann. Rev. Microbiol.* **7**, 273.

Ray, H. N. & Sen Gupta, P. C. 1954. A cytochemical study of *Entamoeba histolytic J. Indian Med. Ass.* **23**, 529.

Rees, C. W. 1929. Pathogenesis of intestinal amebiasis in kittens. *Arch. Path.* **7**, 1.

Rees, C. W., Baernstein, H. D., Reardon, L. V. & Phillips, L. 1953. Some interactio *in vitro* of *Endamoeba histolytica* and single species of microbial symbionts. Ar *J. Trop. Med. Hyg.* **2**, 1002.

Reichenow, E. 1931. Die pathogenetische Bedeutung der Darmprotozoen d Menschen. *Zbl. Bakt.* (I. Orig.) **122** (Ber. 14 Tag. Deutsch. Ver. Mikrobiol.: 19

Ridley, D. S. & Schofield, F. D. 1957. The comparative pathogenicity of small ra *Entamoeba histolytica* and other intestinal parasites. *Trans. R. Soc. Trop. Me Hyg.* **51**, 514.

Simić, T. 1935. Infection expérimentale du chat et du chien par *Entamoeba dispar Entamoeba dysenteriae.* Réinfection et immunité croisée du chien. *Ann. Parasit* **13**, 345.

Swartzwelder, J. C. & Avant, W. H. 1952. Immunity to amebic infection in do *Am. J. Trop. Med. Hyg.* **1**, 567.

Valentino, L. 1956. La reazione di immobilizzazione nell'amebiasi. *Riv. Ist Sieroterap. Ital.* **31**, 310.

lker, E. L. & Sellards, A. W. 1913. Experimental entamoebic dysentery. *Philippine . Sci.* **8B**, 253.
stphal, A. 1937. Betrachtungen und experimentelle Untersuchungen zur Virulenz er *Entamoeba histolytica* beim Menschen. *Arch Schiffs.-u. Tropen-Hyg.* **41**, 262.
stphal, A. 1938. Die Pathogenese der Amöbenruhr beim Menschen. *Arch. Schiffs.- . Tropen-Hyg.* **42**, 441.
stphal, A. 1948. Zur Epidemiologie und Pathogenese der Amöbenruhr in Norda- rika 1941/42. *Z. Hyg.* **128**, 73.
stphal, A. 1950. Die Amöbenruhr. In Gundel's *Die ansteckenden Krankheiten.* 4th d. **1**, 602.
odcock, H. M. 1917. Protozoological experiences during the summer and autumn f 1916. *J.R. Army Med. Corps.* **29**, 290.

STATUS OF BEHAVIORAL AND PHYSIOLOGICAL "RESISTANCE"*

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THE study of acquired humoral resistance to infectious agents ha received just attention in the past 50 years. The antigen-antibod concept has dominated a considerable portion of our thoughts and experi ments on resistance of animals and has reached a high state of mutation in the field of immunochemistry. We have tended to neglect detailed analyses of many phenomena of host-parasite incompatibility which hav their origins in certain other qualities of hosts and parasites.

Dubos (1954) has pointed out that present-day literature usually de scribes disease as a conflict, one party being an "invader" and the othe using "defense mechanisms." We are accustomed to thinking in terms o the resistance or susceptibility of the host to the parasite. However, th host and the parasite may be regarded as an entity, and we may think in terms of those factors which act to prevent the establishment of this en tity. In these terms, resistance and susceptibility will obviously involv attributes of the parasite or the host, or, as is more often the case, both and would embrace that perennially favored subject for speculation, hos specificity.

Schneider (1951) argued cogently that we should distinguish sharpl between resistance and susceptibility. These terms were operationally de fined from experience with nutritional factors: those factors in the nu trition which when withheld from the host decrease the extent or effec of an infection and when supplied increase the effect were termed "sus ceptibility factors." Those nutritional factors which when withheld in crease the extent of infection, and when supplied decrease the effect were termed "resistance factors." I agree with Schneider's suggestion tha we are dealing with separable phenomena and his choice of terms seem reasonable. However, the definitions, as presented by Schneider, are use ful only in considering the relation of nutrition to disease. It seems to m that we may conveniently deal with resistance and susceptibility as sepa rate concepts in more general terms. What we are truly dealing with i

* Some of the work described in this paper has been aided by a contract betwee the Office of Naval Research, Department of the Navy, and Johns Hopkins Universit (NR 119-353) and by a grant, E-1508, from the National Institutes of Health, U.S Public Health Service.

e case of susceptibility are those aspects of the environment furnished
the host which are not directly dependent on previous or present oc-
ıpation by the parasite. In other words, susceptibility is a physiological
ate of the host in which the parasite is supplied with its life needs and
susceptibility is the state in which these life needs are not satisfied,
ither state involving a host response to the parasite. There would obvi-
ısly be intergrading conditions.

Resistance may be defined as those alterations of the physiological
ate of the host which represent a response to previous or present ex-
rience with the parasite or a chemically related entity. In this category
ıtibody production would, of course, be included. Clearly in an indi-
dual host we may have both susceptibility and resistance. By these
finitions we may have a highly susceptible host which is also a highly
sistant one.

In preparing the following discussion, an attempt has been made to
scard resistance, as defined above, from consideration. At the same
ne, some attention has been given to some partners in parasitism,
dinarily falling in the jurisdiction of plant pathology. The latter course
action seemed desirable because of what the present writer considers
be an unjustifiably complete separateness of students of plant and
ımal disease phenomena. The disadvantages of this separateness are
adily seen in the history of the sciences of infectious disease. Prévost
807) demonstrated conclusively that wheat bunt is an infectious disease
ıd that the etiological agent is a microorganism. He devised a chemo-
erapy for the disease and distinguished between fungicidal and fun-
static effects. Comparable work with animals was not forthcoming for
most 40 years. It may be significant that Pasteur's studies on infectious
seases in animals developed from his studies on the "diseases" of non-
ological materials, beer and wine.

In this paper I shall attempt to present evidence to support the general
ncept that insusceptibilities to the attainment and maintenance of
rasitisms are widely distributed and probably common in nature.
ıe pretense cannot be made that this is a complete review of the exam-
es available in the literature. It may also be added that with a moderate
ort many other examples can probably be discovered in nature.

Internal Factors Influencing Parasitisms

It seems obvious that for the establishment of parasitism, the environ-
ental characteristics in or on a host must be compatible with the life
quirements of the parasite. More than this, if the initial events of
tablishment involve multiple physical or chemical stimuli leading to
e alteration of behavioral or other physiological characteristics of the

parasite, it may be expected that the duration and sequence of these en vironmental stimuli may be important in promoting or preventing estab lishment of parasitism. What is the evidence that specific chemical sub stances, produced by the host and not representing a response to the parasite, interfere with maintenance of parasitism? A number of example are available.

No discussion of this subject should overlook the studies of Dr. Acker and his collaborators on the anti-*Ascaridia* substance in duodenal mucu (see Frick and Ackert, 1948 and earlier papers by Ackert, *et al.*). It wa shown that, with ageing of the chicken, there is an increased productio of a substance associated with duodenal mucus which inhibits the estab lishment and growth of *Ascaridia* in the host or growth of the worm i *vitro*. This substance was found to be soluble in 0.8 percent saline an heat stable. Because of the latter property, it was concluded that th substance is not an antibody. Eisenbrandt and Ackert (1941) showe that mucus from dogs and swine had a lethal effect on *Ascaridia in vitr* and suggested this may have bearing on the host specificity of thi nematode. Further studies on the nematode-inhibiting substances asso ciated with mucus have not been forthcoming. It should be feasible t characterize the material with greater precision.

In collaboration with Dr. Lee Douglas and Mr. John Simmons, I hav recently had an opportunity to study the osmotic properties of certa tapeworms of elasmobranch fishes. Incubation of these tetraphyllidea in salt solutions showed that the worms change in wet weight as function of the salt content of the medium and that chloride is gaine or lost, depending on the concentration in the medium. It was show that the addition of urea to salt solutions prevents an initial imbibitio of water by the worms in all salt solutions used (Figure I). It was fou that the blood and the intestinal contents of the hosts and of fresh collected worms have a very high urea content. In *Calliobothrium vertic latum,* almost 4% of the dry weight of the worm consists of urea. Urea lost from tetraphyllideans when they are incubated in urea-free med and the concentration in the tissues of the worm is a function of the co centration of urea in the suspending medium. Further, it was found th shifts in urea content of worms in artificial media are accompanied b chloride shifts. There is an inverse relationship of tissue chloride to co centration of urea in the medium. This supports an hypothesis that ur plays a role, perhaps an indirect one, in maintaining ionic balance these animals and may be extremely important in determining their ho distribution. During these studies it was also observed that urea, at co centrations below those of the elasmobranch gut fluids, is highly toxic several species of tapeworms from birds, mammals, and fishes. The tox

ffects were found to be irreversible. In addition, six species of trematodes rom teleost fishes were rapidly paralyzed in salt solutions containing rea. The data support the conclusion that the high concentration of urea n the elasmobranch gut probably serves as an effective barrier to infec- ion of these hosts by digenetic trematodes or cyclophyllidean and tetra- othriid cestodes (Read *et al.*, 1958).

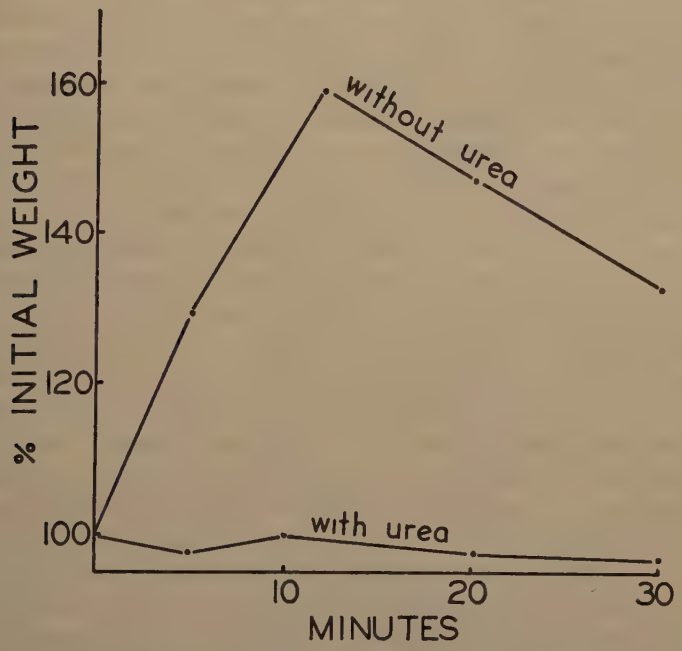

FIG. 1. Changes in the wet weight of *Calliobothrium verticillatum* incubated in lutions with or without urea. The incubations were carried out at room tempera- re. The solutions contained the following per liter: 200 mM NaCl, 4.4 mM KCl, 1 mM $CaCl_2$, and 2.9 mM $MgCl_2$. Urea was added to a concentration of 200 mM er liter.

In a series of studies on the development of pseudophyllidean tape- orms Michajlow has reported that many species of copepods will feed n coracidia. This work has been summarized by Michajlow (1951). It as shown that the fate of such coracidia in the various copepods differed, epending on the action of the digestive fluids. In some copepods, nchospheres of a given pseudophyllidean were killed by the digestive ices. These species of copepods obviously cannot serve as intermediate osts. In other copepods, most of the onchospheres of a given tapeworm e killed by the digestive juices; other things being equal, such species ould serve as what Michajlow called auxiliary hosts. A third group of

copepods secretes digestive juice which seems to have no deleterious effects on the onchospheres. Representatives of the third copepod group may be expected to serve as the usual intermediate hosts. Michajlow's observations are an interesting example of apparent chemical barriers to infection and would merit additional study and confirmation.

Among plant parasites, the classical example of chemical exclusion of a parasite by a potential host is seen in the insusceptibility of some onions to the smudge organism, *Colletotrichum circinans*. This fungus begins its relationship with a host by a saprophytic development on the outer non-living scale of the plant. Eventually it penetrates the underlying living scales and produces pathology. Onion varieties in which the bulb scales are pigmented are highly insusceptible to smudge. Associated with the colored flavone compounds in the dry outer scales are colorless water-soluble phenols, catechol and protocatechuic acid (Link and Walker, 1933). These are toxic to the smudge organism, preventing germination and penetration. That this protection is afforded by the dry outer scales is shown by the fact that if the outer scales are removed and the underlying fleshy scales are exposed to the fungus in a moist chamber, parasitism ensues (Walker, 1923).

Another instance of insusceptibility due to the elaboration of phenolic compounds has been reported by Schaal and Johnson (1955). These authors showed that potatoes which were insusceptible to parasitism by the scab organism, *Streptomyces scabies*, contain high concentrations of chlorogenic acid. Evidence was adduced that when chlorogenic acid is oxidized by tyrosinase the resultant quinones are toxic to the scab organism. Russet color in potatoes is genetically linked to scab-insusceptibility, presumably because the color is due to the presence of the colored quinones.

Beck (1957) studied the role of growth-inhibiting chemical factors in the insusceptibility of corn to the establishment and survival of the European corn borer, *Pyrausta nubilalis*. The total quantity of growth-inhibiting substances in the tissues of various inbred corn strains was determined by bioassay, using corn borers maintained on purified diets. The total inhibitor activity, as determined by bioassay, was found to be in agreement with susceptibility ratings of the particular strains as determined in field tests. It was shown that three antiparasitic chemical entities are involved: Factor A (6-methoxy-benzoxazolinone) was found to be important in the insusceptibility of early plant growth stages, but negligible after the development of the visible corn tassel. The chemically-undefined Factors B and C contributed about equally to insusceptibility of the internode, leaf sheath, husks, and silk tissues. Virtanen and Hietala (1955) reported that insusceptibility to the fungus, *Fusarium*, in rye could be

ttributed to the presence of a fungus-inhibiting substance related to actor A, 2(3)-Benzoxazolinone, in the seedlings. Virtanen, *et al.* (1956) howed that fungus-insusceptible corn contained Factor A.

It may be noted that some of Beck's borer-insusceptible corn strains ere originally selected for insusceptibility to stalk-rot, a fungus disease, nd were subsequently found to be borer insusceptible. Johann and ickson (1945) had described the presence in corn of an inhibitor of *iplodia,* the stalk-rot organism. The inhibitor is apparently identical ith Factors A or C involved in insusceptibility to the corn borer. This of great interest since what might first appear to be genetic linkage f parasite-inhibiting factors resolved into a common causal basis of nsusceptibility to parasites of different kingdoms.

A subtle form of insusceptibility is suggested by recent work on the ffects of bile salts on the metabolism of the strobilae of certain tape-orms (Rothman and Read, 1957; Rothman, in press). In the presence f physiological concentrations of bile salts, the metabolism of *Hymeno-epis* and *Oochoristica* was inhibited to a much greater extent at pH 7.0 han at pH 7.4. If this is considered in terms of the complications of ntestinal physiology, it is readily seen that effective inhibition by bile alts may occur in some hosts and not in others. Although they have been eviewed elsewhere (Read, 1950), salient points may be indicated: .) There is a linear hydrogen-ion gradient in the small intestine, the pH ncreasing from anterior to posterior. (2) There is a linear bile-salt-oncentration gradient, the sites of absorption differing with the quality f bile salt. (3) The kinds of bile salts secreted are phylogenetically dif-erent. (4) The anatomical position of the intestinal orifice of the bile ucts shows considerable variation among different species and would be xpected to condition the extent and nature of the gradients mentioned bove. Thus it may be expected that, in some hosts, these variables will esult in environments which are incompatible with survival of these orms.

There is evidence that the action of specific intestinal secretions may e of importance in the decystment and evagination of tapeworm larvae. arious workers have reported that bile or bile salts cause evagination f tapeworms. Malkani (1933) produced rapid evagination of cysticerci f *Taenia tenuicollis* and *T. saginata* by treating them with diluted bile *vitro.* DeWaele (1934) made similar observations with *T. pisiformis.* oth Malkani and deWaele noted that the bile salts, glycocholate and urocholate, had different effects on *Taenia* spp. Malkani noted that *T. nuicollis* evaginated more slowly in glycocholate than in taurocholate. eWaele reported that glycocholate is toxic for *T. pisiformis,* whereas e worm readily tolerates taurocholate. DeWaele postulated that this

effect explains the fact that *T. pisiformis* occurs only in hosts (dogs) having taurocholate and lacking glycocholate as a bile constituent.

In addition to these examples of substances which may interfere with parasitism by a directly deleterious effect on the parasite, there are cases in which the establishment of parasitism may not occur because of the disoperation of stimuli. When multiple stimuli are involved in the establishment of a parasitism the sequence and timing of stimuli may be important. It has been shown that intestinal emptying time may be a critical factor in determining the degree of establishment of *Hymenolepis nana* (Larsh, 1947) and *H. diminuta* (Read and Voge, 1954). When timing is compounded with other required factors, considerable specificity of effect may ensue. Read (1955) attempted a quantitative study of the factors involved in the excystment of the cysticercoid of *Hymenolepis diminuta*. It was postulated that a complex of five factors was involved. These were a priming effect of gastric juice, specifically pepsin at low pH, an excitatory effect of bile salts, a denaturation effect of bile salts on the proteins of the larval cyst, a proteolytic effect of trypsin, and an effect of elevation of temperature. It was shown that the final result, excystment, depended on the interactions of these factors. Rothman (1958) has extended these findings and has made a comparative study of the effects of intestinal secretion components on cysticercoids of *Hymenolepis diminuta, H. citelli, H. nana,* and *Oochoristica symmetrica,* and on the strobilocercus of *Taenia taeniaeformis.* He found rather marked quantitative differences in the rates at which the *Hymenolepis* spp. respond to these secretions. There was a characteristic maximum time of treatment in gastric juice beyond which further treatment had negligible effects; *H. diminuta* excysted most rapidly after a treatment of at least 30 minutes whereas *H. nana* responded maximally after 15 minutes' treatment. The cysticercoid of *H. nana* excysted about twice as rapidly in a solution containing trypsin and bile salts as *H. citelli* and *H. diminuta* if the worms had not been pretreated with artificial gastric juice. The rate of excystment of *H. citelli* larvae was slower in old larvae than in young ones; ageing was without effect on the rate of response of *H. diminuta* larvae. The excitatory response to bile salts appeared much more slowly in *H. citelli* than in *H. diminuta* cysticercoids. *Oochoristica* was found to be deleteriously affected by gastric juice and readily excysted in salt solutions containing bile salts at low concentrations. With *Oochoristica,* a definite optimum concentration of bile salts was observed; at concentrations above the optimum the worms evaginated but reinvaginated on continued exposure. *Taenia taeniaeformis* required treatment with pepsin before evagination would occur upon subsequent treatment in salt solutions which were neutral or slightly basic in reaction. Bile salts were not re-

quired for evagination of *T. taeniaeformis* and, at concentrations above 0.1%, bile salts caused a reinvagination of the strobilocercus. The known qualitative effects on tapeworm larvae of gastric juice, bile salts, and trypsin are summarized in Table 1. Rothman has reported a number of other quantitative differences between closely related species. Each species studied has been found to require interacting factors for excystment, and these differ considerably between species. There are, of course, a number of other untested variables which may have effects. However, the observations available support the hypothesis that the quality, quantity, and sequence of factors involved in the excystment of tapeworms may play an important role in determining whether a host is resistant to the initial establishment of a particular cestode species.

TABLE I

	Treatment in Gastric Juice	Effects of Bile Salts		Effect of Trypsin
		(Glycocholate)	(Taurocholate)	
Taenia				
pisiformis	0	(lethal)	++	0
tenuicollis	0	+	++	0
taeniaeformis	+	(0; reinvagination at high concentrations)		0
Hymenolepis				
nana	0	+	+	0
citelli	+	+	+	+
diminuta	+	+	+	+
Oochoristica				
symmetrica	(lethal)	+	+	0

The differential effects of certain gastrointestinal factors on excystment of tapeworm larvae *in vitro*. Data from Malkani (1933), deWaele (1934), Read (1955), Rothman (1958). 0=not required or without effect; +=required or effective; ++=marked effect.

There are other evidences that in some cases physiological incompatibility may be restricted to a particular step in the life cycle. *Echinococcus granulosus* and *E. multilocularis* furnish pertinent examples. The former species develops as the well known hydatid cyst in large mammals such as sheep, pig, and man but is apparently incapable of developing in rodents. On the other hand, *E. multilocularis* develops in certain rodents when these animals ingest the eggs of the worm. Schiller and Read (1958) have recently shown that there is no apparent lack of ability of rodent tissues to support the development of *E. granulosus*. Living cysts of *E. granulosus* removed from man at surgery were broken up in a blender for 10 seconds. This material was injected intraperitoneally in hamsters, mice, rats, and a monkey. Growth occurred in all these hosts. Parentheti-

cally, it may be emphasized that this form has not developed in a multilocular pattern in these animals. These observations strongly support the hypothesis that the failure of *E. granulosus* to infect rodents when these animals ingest eggs must be due to a failure of the early larvae to invade the tissues. It would be extremely interesting to study the comparative physiology of onchosphere liberation in these two species, since this may be the determining factor in intermediate host distribution.

These observations on *Echinococcus* are reminiscent of the studies of *Taenia pisiformis* reported by Leonard and Leonard (1941). These investigators found that if the onchospheres of *T. pisiformis* were inoculated into the mesenteric vein, there was no significant difference in the number of young developing larval worms in resistant and control rabbits. Leonard and Leonard concluded that the intestinal mucosa serves as an important barrier in preventing the onchospheres from reaching the vascular system in resistant hosts. However, we are dealing with qualitatively different phenomena in the cited examples of *Echinococcus* and *Taenia.* In one case, we are probably dealing with an insusceptibility and in the other with an acquired resistance. The basis for the insusceptibility may be an absence of stimuli necessary for onchosphere hatching and/or penetration. That of the acquired resistance may be the presence of inhibitory substances or cellular reactions which interfere with the hatching and/or penetration of the onchosphere. These possibilities should merit experimental study.

My colleague, Dr. Laurie, (1958) has studied the capacity of a variety of metabolites to induce encystment of the cercaria of the trematode, *Himasthla quissetensis.* In its life cycle this species encysts in certain bivalve molluscs (Stunkard, 1938). Laurie observed that some specificity was evident in the induction of encystment by different chemical substances. Encystment was induced by the following compounds, in order of relative activity: lysine, glucose, cellobiose, alpha-methylglucoside, galactose, gluconic acid, maltose, glutamic acid, mannose, glucosamine, xylose, arginine, and 3-0-methylglucose. Glucose and glutamic acid together produced an additive effect. A casein hydrolysate was more effective than any of the single compounds tested and the activity of the hydrolysate was enhanced by the addition of glucose. On the other hand, the following compounds did not induce encystment, during periods of exposure up to 24 hours: fructose, sorbose, 2-deoxyglucose, arabinose, rhamnose, trehalose, sucrose, lactose, melibiose, raffinose, glucosyloxime, dulcitol, sorbitol, i-inositol, mannitol, glucuronic acid, mucic acid, glycerophosphate, succinate, citrate, malonate, lactate, beta-alanine, glycine, methionine sulfoxide, DL-threonine, L-tyrosine, yeast nucleic acid, acetamide, and urea. Significantly, beta-alanine was found to antagonize the

induction of encystment by lysine. These observations indicate that there may not only be some specificity in the capacity of intermediate host secretions to induce encystment of *Himasthla* cercaria but that the presence of a specific substance in such fluids may inhibit encystment even in the presence of stimulatory compounds. Investigation of this hypothesis seems immediately feasible.

Recent studies by Sommerville (1957) and Rogers and Sommerville (1957) on the physiology of exsheathment of trichostrongyle nematode larvae may prove to be of great importance in understanding insusceptibility of some hosts to certain nematode species. These workers have presented evidence that there is a chemical stimulation afforded by host secretions which triggers the secretion by the nematode of substances causing exsheathment. It was shown that the exsheathing stimulant in host secretions is dialysable (in part) and heat-labile, and that its activity is affected by hydrogen ion concentration and oxidation-reduction potential. Optima for the former physical factor differed with the nematode species tested. The data suggest that there are a sufficient number of interdependent variables involved to expect that exsheathment would not occur in all animals *at sites allowing the survival of the fourth stage.* This assumes significance above the speculative level in view of the observation of Madsen and Whitlock (personal communication) that, in sheep showing natural insusceptibility to *Haemonchus,* the number of larvae exsheathing is markedly less than in genetically susceptible sheep.

Alteration of Susceptibility by External Factors

There is literature dealing with the relationships between nutrition and parasitism. These studies have for the most part been concerned with medical and veterinary problems and the emphasis has most often been on the aggravation of malnutrition by parasitism or vice versa. However, it has been pointed out that state of nutrition may interfere with the establishment of parasitism (Chandler, 1957). A few instances may be cited: Some years ago, Reiner and Paton (1932) showed that when rats are fed on diets deficient in B-complex vitamins, the growth of *Trypanosoma equiperdum* is inhibited. As far as I can ascertain, no further studies of this have been made. Seeler and Ott (1944) showed that the feeding of a riboflavin-deficient diet interfered with the multiplication of *Plasmodium lophurae.* There has been a considerable amount of recent interest in the finding that a low intake of para-aminobenzoic acid by the host suppresses the growth of *Plasmodium knowlesi* (Geiman and McKee, McKee and Geiman, 1948) and *P. berghei* (Maegraith, *et al.* 1952; Hawking, 1954; Fulton and Spooner, 1955; and others). The latter parasite seems to be adversely affected by a variety of environmental alterations,

including the dietary deficiencies (see above), pituitary somatotrophic hormone (Galliard, *et al.*, 1954), or the presence of another infectious agent (Adler, 1954). In some of these cases, additional study is required to determine whether there is an alteration of susceptibility or resistance or both.

Reducing agents in the host diet have been found to alter the course of certain parasitisms. McKee and Geiman (1946) showed that a deficiency of dietary ascorbic acid reduces the numbers of *Plasmodium knowlesi* in monkeys although, *in vitro*, lack of ascorbic acid in the medium had no appreciable effect on parasite multiplication. Godfrey (1957a) showed that the addition of cod liver oil to a stock diet suppressed the growth of *Babesia rodhaini*. Addition of vitamins A and D did not cause suppression of the piroplasm, but the unsaturated fatty acid fraction of cod liver oil was a highly effective suppressant. The cod-liver-oil suppression was reversed by adding ascorbic acid, vitamin E, or tetraethyl thiuram disulfide to the host diet. Addition of cod liver oil to the host diet was also shown to suppress the growth of *Plasmodium berghei*. This suppression was also antagonized by the addition of antioxidants to the diet (Godfrey, 1957b). In explanation of his observations, Godfrey suggested that the unsaturated fatty acids of cod liver oil are oxidized in the host's tissues to peroxides which inhibit development of the parasite. The reversal of the effect by antioxidant compounds strongly supports Godfrey's hypothesis. Greenberg *et al.* (1954) reported that feeding of maize oil, butter fat, and the fatty fraction from dried milk suppressed *Plasmodium gallinaceum* infection in chicks. These substances do not have similar effect when fed to mice with *P. berghei* infections (Godfrey, 1957b). Keppie (1953) reported that feeding mice diets composed of starch, salts, dried yeast, margarine, cod liver oil, and varying quantities of casein to mice inoculated with *Trypanosoma congolense* resulted in infections of low intensity or no infection at all. Godfrey has carried out further studies on effects of diets containing cod liver oil on infections with *Trypanosoma* spp., but these studies have not yet been published (Godfrey, 1957a).

The apparently contradictory nature of evidence for and against an ascorbic acid requirement by trypanosomes (reviewed by Lwoff, 1951) may be due to indirect effects of a similar type. (Lwoff has not discussed this evidence objectively. For example, the statement, ". . . it is well known that Trypanosomidae are strict aerobes . . ." is not justified by published information, and Mr. R. Zeledon, in our own laboratory, is currently growing several species of hemoflagellates in anaerobic culture. While it is true that some hemoflagellates are obligate aerobes, some are not.)

Whitlock (1949) reported that, when sheep were given supplementary

food, there was an apparently increased resistance to trichostrongylids in the small intestine but an apparently decreased resistance to *Haemonchus*. Experimental animals on a nutritionally optimum diet had more anemia and more *Haemonchus* (three times as many) than pasture-fed animals. This is probably a case in which a marked increase in susceptibility overrides a lesser increase in resistance. Since *Haemonchus* produces more pathology than the other sheep trichostrongylids, the practical importance of these observations is obvious.

Beaver (1937) probably observed a susceptibility alteration when he found that pigeons become refractory to infection with *Echinostoma revolutum* when maintained on a laboratory diet of yellow corn, with some wheat, oats, and rice added. Many workers have had the experience of bringing a wild animal into the laboratory and sorrowfully watching it lose its parasites. In some cases this may be due to the unavoidable alterations of host nutrition, with concomitant alterations of susceptibility.

During the past two years, my colleagues and I have published a series of studies on the role of carbohydrates in the biology of cestodes. These and related studies of others have been summarized and appropriate conclusions drawn (Read, 1958). It was concluded that (1) tapeworms require carbohydrates for growth, maintenance of size, and reproduction; (2) the carbohydrate requirements of the worms are satisfied by utilization of carbohydrate ingested by the host before such material has been absorbed by the host; (3) most tapeworms are capable of utilizing a few monosaccharide sugars but are incapable of utilizing di- or polysaccharides; (4) alteration of the quality of carbohydrate in the host diet results in reduction or increase in worm size, growth rate, egg production, and, in some instances, initial establishment of tapeworms; (5) the effects of host dietary carbohydrates of differing quality on tapeworms may be explained in terms of the physiology of the gut. It is plain that, in nature, selection of food of particular quality in carbohydrate content may play a part in determining whether and to what extent a parasitism of a particular worm species and a particular host species will be maintained.

The plant pathologists have amassed a considerable quantity of information on the effects of altering external environmental factors on the course of parasitisms. Such factors are potassium, nitrogen, or phosphorus content of the soil, pH, temperature, and humidity. Alterations of one or all of these factors may determine whether there will be parasitism of a plant in the presence of bacteria, fungi, nematodes, or viruses (Walker, 1957). It seems likely that in most cases these involve alterations of susceptibility. Incidentally, resistance also occurs in plants. For

example, the cellular reaction of certain strains of wheat to the fungus *Puccinia,* results in the death of the parasite (Allen, 1923). However, antibody formation has not been demonstrated in plants, and the information on cellular responses has been gained primarily in connection with studies of pathology *per se.*

There are instances which clearly suggest that different behavior patterns of sympatric host species may be involved in determining the distribution of parasites. For example, Read and Millemann (1953) found *Oochoristica deserti* Millemann in *Dipodomys merriami merriami* and *Citellus leucurus* but not in *D. panamintinus mohavensis* trapped in the same locality. Millemann (1953, 1955) corroborated this with more extensive trapping data and showed that although *D. panamintinus mohavensis* does not harbor *Oochoristica* in nature it is readily infected with this tapeworm in the laboratory. This might represent an example of host insusceptibility which is behavioral. There are alternative behavioral hypotheses: *D. panamintinus mohavensis* may eschew the eating of the intermediate host or this mammal may choose items in its natural diet which render its gut unfit as a habitat for *Oochoristica.* Another example which may be cited involves studies which are still in progress in our laboratory. These studies have been carried out in collaboration with Drs. E. L. Schiller, D. E. Davis, and V. Flyger. Schiller (1958) showed that the gray squirrel, *Sciurus carolinensis,* was readily infected with *Hymenolepis nana* in the laboratory. These animals were fed on dog biscuits. As a matter of fact, *H. nana* develops more rapidly to a larger size, lives longer and produces more eggs in *Sciurus* than in its "natural host," the mouse. However, *H. nana* has never been reported as a parasite of wild-caught *Sciurus.*

It seemed feasible to introduce this parasite into a previously studied, isolated, wild population of gray squirrels and determine the course of the infection. This was done, 10% of the population being infected in the field. Recaptures over a period of several months showed that the infection did not spread in this population and, as a matter of record, *did not develop significantly in those hosts initially infected.* This strongly suggested that the feeding habits of squirrels may result in an intestinal environment which is not at all suitable for *H. nana.* This has been tested in the laboratory by furnishing infected captive squirrels with various foods which they have been observed to utilize in nature. The results support the hypothesis that on certain diets the squirrel is insusceptible. This work will be reported in detail elsewhere. This seems to furnish an excellent example of a species which is insusceptible to a particular parasite by virtue of the foods which are chosen under natural conditions. I feel reasonably confident that if a wild population of *Sciurus* were fed

on dog biscuits, *H. nana* could readily establish itself in this host under natural" conditions. We are attempting to test this experimentally by urnishing a wild population with dog biscuits.

In considering behavioral aspects of susceptibility, the behavior of he parasite should receive attention. The relationships of behavior to pecificity in symbiosis have been reviewed recently by Davenport (1955). The conclusion seems inescapable that chemotropic responses of symbiotes to their hosts are widespread and, in some cases, sufficiently specific o account for the host distribution observed in nature. Painstaking comarative study of the chemotropic responses of the free-living stages of ome parasites might shed light on certain problems of host and geographical distribution. For example, there is evidence that the miracidium f *Schistosoma japonicum* shows chemotactic responses to a specific snail nd its mucus tract (Faust and Meleney, 1924). On the other hand, *asciola hepatica* miracidia show highly non-specific chemotropic responses (Neuhaus, 1953). Are these properties merely coincidental with he fact that *Fasciola hepatica* has an essentially cosmopolitan, though potty, distribution, whereas *Schistosoma japonica* is restricted in its istribution to certain areas of the Orient? The specific behavioral responses of parasites to chemical stimuli derived from potential hosts have ot received enough consideration from parasitologists.

Sociopsychological Stresses and Resistance

In spite of my stated effort to delete antigens and antibodies from this iscussion, some mention must be made of an aspect of resistance which ay involve behavior and certain concomitant effects on the capacity of ost tissues to interfere responsively with establishment and maintenance f parasitism. There is a considerable body of evidence that, in the mamal, stresses of various types stimulate the release of adrenocorticotrophic ormone from the anterior pituitary via pathways involving the higher rain centers and the hypothalamus, with an increase in production of drenal glucocorticoids (Harris, 1956). There is evidence that the injection f adrenal corticoids decreases the inflammatory responses of vertebrate issues to foreign proteins or infectious agents. It has been shown that his is accompanied by an apparent loss of resistance to a number of arasitic organisms, such as viruses (Schwartzmann, 1950), bacteria Berlin *et al.*, 1952), yeasts (Seligmann, 1953), rickettsiae (Whitmire and Downs, 1954), and helminths (Stoner and Godwin, 1953, 1954; Weinstein, 953, 1955; Coker, 1955, 1956 a, b, c). As might be anticipated, stresses nduced a suppression of inflammatory responses in the vertebrate, and esistance to foreign biochemical stimuli in general are depressed (Selye, 952).

During the past 6 years evidence has accumulated in support of the concept that population dynamics of mammals, specifically fluctuations in numbers, are governed to a considerable extent by sociopsychological stresses. The evidence has recently been reviewed by Christian (1958) and will not be reviewed in detail here. The pertinent evidence indicates that, with increasing density of population, resulting psychogenic stresses produce increased activity of the pituitary-adrenocortical system. This is not referable to lack of food, water, nesting space, nor materials for nest-building but is attributable to social competition which can be defined in "units of social pressure." Thus, the stress of increasing contact with individuals of the same species results in an increase in the activity of the adrenal cortex, as well as inducing other effects on gonadal physiology. This leads us to offer the hypothesis that sociopsychological stresses, such as crowding, may produce changes in the physiology of the mammal which result in increased adrenal activity and a lowering of the tissue responses which interfere with the establishment or extent of parasitism. To my knowledge, not a single experimental test of this probability has been published. Dr. D. E. Davis and I are currently carrying out experimental work to test the hypothesis which was arrived at through the collaborative thinking of Dr. J. J. Christian, Dr. Davis, and, to a minor extent, myself. This hypothesis furnishes diverse ramifications. We may see that the physiological opportunities for establishment of parasitism would involve a variable which is independent of the variable of ecological opportunity. We may visualize the possibility that the establishment of parasitism involving new host-parasite combinations may be a function of population density, exclusive of the probabilities of transmission at varying densities. It is easy to see why this may have been overlooked previously in wild populations. Similar conditions representing social stresses may have direct effects on the course of parasitism; the obvious example is the enhancement of contagion with increasing population densities.

Further, it is important to point out that other stresses may be important in this connection. Malnutrition, for example, has been shown to serve as a stress which increases adrenocortical activity (Selye, 1946). One wonders if the many forms of malnutrition which have been shown to alter the character of parasitisms in laboratory animals and are thought to produce "disoperations" in parasitisms of human populations (Chandler, 1957) may not produce some of their effects through pituitary-adrenocortical stimulation, with resultant depression of resistance responses, rather than through direct depletion of the antibody-synthesizing systems.

There are possibilities for practically unlimited speculations and questions on the roles of population size, position of the individual in the social hierarchy, populations of other species, and other features of the environment acting as stressors through the host's endocrine system to modify parasitisms. I shall not indulge in further speculation on the point, but it would seem that this may be a very fertile field for investigation.

In dealing with problems involving alterations of innate susceptibility of hosts to disease agents the term "mutation" usually enters the discussion. In the case of vertebrates we may reasonably expect that alterations of genetic material will underlie alterations of innate susceptibility. This will certainly be true in those instances involving biochemical susceptibility whether this be due to the presence of an inhibitory substance or absence of a required substance. It may be pointed out that this will also be true of behavioral susceptibility. The genetic basis of vertebrate behavior has been pointed out by various authors (Tinbergen, 1953) and is frequently considered to be an important mechanism of isolation (Huxley, 1942). Thus, acquisition of behavioral insusceptibility might be expected to require alteration of genetic makeup. A most interesting exception to this is man. Non-genetic alterations of human behavior having the same significance for the species that genetic mutations may have for other vertebrates are readily recognizable. Some authors (e.g. Linton, 1955), have referred to the use of fire, the wheel, etc., as "mutations." In this same sense, the use of DDT, antibiotics, sewage disposal, and such may be regarded as alterations of behavioral resistance of the species without concomitant genetic alteration. It is interesting to point out that in man non-genetic alterations of behavioral susceptibility lead to alterations of age composition of populations and may conceivably lead to alterations of genetic composition, putting the shoe on the other foot. The role of non-genetic behavioral mutations in the isolation of human populations has interesting implications well outside the scope of the present discussion.

In summary, it may be said that the study of physiological and behavioral susceptibilities to animal parasites is in a primitive state. The foregoing discussion may contribute little to general understanding of the subject, but it is earnestly hoped that it may interest others in experimental consideration of the multitude of problems at hand. We may feel a bit like Dr. E. Racker's drunkard who lost the key to his door and searched for it under the street lamp where the light was more favorable for the search. Dr. Racker's friend found a key, but in his drunkenness he could not determine if it was his own. But what a fine time he had, sitting on his doorstep, dreaming of all the castles it might open.

REFERENCES

Adler, S. 1954. The behavior of *Plasmodium berghei* in the golden hamster *Mesocricetus auratus* infected with visceral leishmaniasis. *Trans. Roy. Soc. Trop. Med. Hyg.* **48**, 431-440.

Allen, R. F. 1923. Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis* forms III and XIX. *J. Agr. Res.* **26**, 571-604.

Beaver, P. B. 1937. Experimental studies on *Echinostoma revolutum* (Froelich) a fluke from birds and mammals. *U. Ill. Biol. Monog.* **15**, 1-96.

Beck, S. D. 1957. The European corn borer, *Pyrausta nubilalis* (Hübn.), and its principal host plant—VI. Host plant resistance to larval establishment. *J. Insect Physiol.* **1**, 158-177.

Berlin, B. S., Johnson, C., Hawk, W. D., and Lawrence, A. G. 1952. The occurrence of bacteremia and death in cortisone-treated mice. *J. Lab. Clin. Med.* **40**, 82-89.

Chandler, A. C. 1957. Interrelations between nutrition and infectious disease in the tropics. *Am. J. Trop. Med. Hyg.* **6**, 195-208.

Chitty, D. 1954. Tuberculosis among wild voles with a discussion of other pathological conditions among certain mammals and birds. *Ecology* **35**, 227-237.

Christian, J. J. 1958. A review of the endocrine responses in rats and mice to increasing population size including delayed effects on offspring. In *Am. Assoc. Adv. Sci. Symp. on Research of Sociological Significance.* In press.

Coker, C. M. 1955. Effects of cortisone on *Trichinella spiralis* infections in non-immunized mice. *J. Parasitol.* **41**, 498-504.

Coker, C. M. 1956. Cellular factors in acquired immunity to *Trichinella spiralis*, as indicated by cortisone treatment of mice. *J. Inf. Dis.* **98**, 185-197.

Coker, C. M. 1956. Some effects of cortisone in mice with acquired immunity to *Trichinella spiralis. J. Inf. Dis.* **98**, 39-44.

Coker, C. M. 1956. Effects of cortisone on cellular inflammation in the musculature of mice given one infection with *Trichinella spiralis. J. Parasitol.* **42**, 479-484.

DeWaele, A. 1934. Étude de la fonction biliare dans le phénomène de l'evagination chez les cysticerques des cestodes. *Ann. Parasitol.* **12**, 492-510.

Dubos, R. J. 1954. *Biochemical Determinants of Microbial Diseases.* Harvard U. Press, Cambridge. 152 pp.

Eisenbrandt, L. L., and Ackert, J. E. 1941. Effects of duodenal mucus of dogs and swine upon the viability of *Ascaridia lineata* in vitro. *J. Parasitol.* **27** (Suppl.), 36.

Faust, E. C., and Meleney, H. E. 1924. Studies on schistosomiasis japonica. *Am. J. Hyg. Monogr.* **3**, 339 pp.

Frick, L. P., and Ackert, J. E. 1948. Further studies on duodenal mucus as a factor in age resistance of chickens to parasitism. *J. Parasitol.* **34**, 192-206.

Fulton, J. D., and Spooner, D. F. 1955. The biochemistry and nutrition of *Plasmodium berghei. Indian J. Malariol.* **9**, 161-176.

Galliard, H., Lapierre, J., and Murard, J. 1954. Inhibition de l'infection à *Plasmodium berghei* chez la souris et le rat par l'hormone hypophysaire de croissance (S.T.H.). *Ann. Parasitol. Hum. Comp.* **29**, 167-178.

Geiman, Q. M., and McKee, R. W. 1948. Malarial parasites and their mode of life. *Sci. Monthly* **67**, 217.

Godfrey, D. G. 1957a. The influence of dietary cod liver oil and vitamin E upon *Babesia rodhaini* in mice. *Exp. Parasitol.* **6**, 465-485.

Godfrey, D. G. 1957b. Antiparasitic action of dietary cod liver oil upon *Plasmodium berghei* and its reversal by vitamin "E." *Exp. Parasitol.* **6**, 555-564.

Greenberg, J., Taylor, D. J., and Trembley, H. L. 1954. The effect of milk diets on the course of sporozoite-induced *Plasmodium gallinaceum* infections in chicks. *Am. J. Hyg.* **60**, 99-105.

Harris, G. W. 1956. Hypothalamic control of the anterior lobe of the hypophysis. In *Hypothalamic-hypophyseal interrelationships,* Ed. W. S. Fields, R. Guillemin, and C. A. Carton. Chas. C Thomas, Springfield.

Hawking, F. 1954. Milk, p-aminobenzoate, and malaria of rats and monkeys. *Brit. Med. J.* **1**, 425-429.

Huxley, J. 1942. *Evolution the Modern Synthesis.* Harper, N.Y. 645 pp.

Johann, H., and Dickson, A. D. 1945. A soluble substance in cornstalks that retards growth of *Diplodia zeae* in culture. *J. Agr. Res.* **71**, 89-110.

Keppie, A. N. 1953. Modified course of *Trypanosoma congolense* infection in mice given diets with milk casein. *Brit. Med. J.* **2**, 853-867.

Larsh, J. E., Jr. 1947. The relationship in mice of intestinal emptying time and natural resistance to *Hymenolepis. J. Parasitol.* **33**, 79-84.

Larsh, J. E., Gilchrist, H. B., and Greenberg, B. G. 1952. A study of the distribution and longevity of adult *Trichinella spiralis* in immunized and non-immunized mice. *J. Elisha Mitchell Sci. Soc.* **68**, 1-11.

Larsh, J. E., Jr., Race, G. J., and Jeffries, W. B. 1956. The association in young mice of intestinal inflammation and the loss of adult worms following an initial infection with *Trichinella spiralis. J. Inf. Dis.* **99**, 63-71.

Laurie, J. S. 1958. Induced *in vitro* encystment of the cercaria of *Himasthla quissetensis.* Unpublished ms.

Leonard, A. B., and Leonard, A. E. 1941. The intestinal phase of the resistance of rabbits to the larvae of *Taenia pisiformis. J. Parasitol.* **27**, 375-378.

Link, K. P., and Walker, J. C. 1933. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. *J. Biol. Chem.* **100**, 379-383.

Linton, Ralph. 1955. *The Tree of Culture.* Knopf, N.Y. 692 pp.

Lwoff, M. 1951. The nutrition of parasitic flagellates (Trypanosomidae, Trichomonadinae). In *Biochemistry and physiology of protozoa* I. Ed. A. Lwoff. Acad. Press, N.Y. pp. 129-176.

Maegraith, B. G., Deegan, T., and Jones, E. S. 1952. Suppression of malaria *(P. berghei)* by milk. *Brit. Med. J.* **2**, 1382-1384; 1405.

Malkani, P. G. 1933. A rapid method of evaginating the scolices in parasitic cysts. *Indian Vet. J.* **10**, 122-124.

McKee, R. W., and Geiman, Q. M. 1946. Studies on malarial parasites. V. Effects of ascorbic acid on malaria in monkeys. *Proc. Soc. Exp. Biol. Med.* **63**, 313-315.

McKee, R. W., and Geiman, Q. M. 1948. Methionine in the growth of the malarial parasite, *Plasmodium knowlesi. Fed. Proc.* **7**, 172.

Michajlow, W. 1951. Stadialnosc rozwoju niektorych tasiemcow (Cestoda). (Uderzajca analogia biologiczna). *Ann. Universit. Mariae Curie-Sklowdowska* **6**, 77-147.

Millemann, R. E. 1953. Studies on the biology of the cestode, *Oochoristica deserti* n. sp. Ph.D. Dissertation, Univ. Calif., Los Angeles.

Millemann, R. E. 1955. Studies on the life-history and biology of *Oochoristica deserti* n. sp. (Cestoda: Linstowiidae) from desert rodents. *J. Parasitol.* **41**, 424-440.

Neuhaus, W. 1953. Über den chemischen Sinn der Miracidien von *Fasciola hepatica. Zeitschr. Parasitenk.* **15**, 476-490.

Prévost, B. 1807. Mémoire sur la cause immédiate de la carie ou charbon des blés, et de plusieurs autres maladies des plantes, et sur les préservatifs de la carie. Paris, 1807. Engl. transl. by G. W. Keitt in *Phytopath. Classics* 6 (1939).

Racker, E. 1954. Metabolism of infected cells. In *Cellular Metabolism and Infections.* Ed. E. Racker. Academic Press, N.Y. pp. 138-148.

Read, C. P. 1955. Intestinal physiology and the host-parasite relationship. In *Some Physiological Aspects and Consequences of Parasitism.* Ed. W. H. Cole, Rutgers Univ. Press. pp. 27-43.

Read, C. P. 1958. The role of carbohydrates in the biology of cestodes. VIII. Some conclusions and hypotheses. *Exp. Parasitol.* In press.

Read, C. P., Douglas, L. T., and Simmons, J. E. 1958. Urea and osmotic properties of tapeworms from elasmobranchs. *Exp. Parasitol.* In press.

Read, C. P., and Millemann, R. E. 1953. Helminth parasites in kangaroo rats. *Univ. Calif. Publ. Zool.* **59**, 61-80.

Read, C. P., and Voge, M. 1954. The size attained by *Hymenolepis diminuta* in different host species. *J. Parasitol.* **40**, 88-89.

Rogers, W. P., and Sommerville, R. I. 1957. Physiology of exsheathment in nematodes and its relation to parasitism. *Nature* **179**, 619-621.

Rothman, A. H. 1958a. Sc.D. Dissertation, Johns Hopkins University.

Rothman, A. H. 1958b. The role of bile salts in the biology of tapeworms. I. Effects of bile salts on the metabolism of *Hymenolepis diminuta* and *Oochoristica symmetrica*. *Exp. Parasitol.* In press.

Rothman, A. H., and Read, C. P. 1957. Effects of bile salts on tapeworm metabolism. *Anat. Rec.* **128**, 611.

Schaal, L. A., and Johnson, G. 1955. The inhibitory effect of phenolic compounds on the growth of *Streptomyces scabies* as related to the mechanism of scab resistance. *Phytopathol.* **45**, 626-628.

Schiller, E. L. 1958. Sc.D. Dissertation, Johns Hopkins University.

Schiller, E. L., and Read, C. P. 1958. Experimental studies on *Echinococcus granulosus*. Ms. in preparation.

Schneider, T. A. 1951. Nutrition and resistance—susceptibility to infection. *Am. J. Trop. Med.* **31**, 174-182.

Schwartzmann, G. 1950. Enhancing effect of cortisone upon poliomyelitis infection (strain MEF1) in hamsters and mice. *Proc. Soc. Exp. Biol & Med.* **75**,835-838.

Seeler, A. O., and Ott, W. H. 1944. Effect of riboflavin deficiency on the course of *Plasmodium* infection in chicks. *J. Inf. Dis.* **75**, 175.

Seligmann, E. 1953. Virulence enhancement of *Candida albicans* by antibiotics and cortisone. *Proc. Soc. Exp. Biol. & Med.* **83**, 778-781.

Selye, H. 1946. The general adaptation syndrome and the diseases of adaptation. *J. Clin. Endocrinol.* **6**, 117-230.

Selye, H. 1951. First annual report on stress. Acta, Inc., Montreal.

Sommerville, R. I. 1957. The exsheathing mechanism of nematode infective larvae. *Exp. Parasitol.* **6**, 18-30.

Stoner, R. D., and Godwin, J. T. 1953. The effects of ACTH and cortisone upon susceptibility to trichinosis in mice. *Am. J. Pathol.* **29**, 943-950.

Stoner, R. D., and Godwin, J. T. 1954. The effects of adrenocorticotrophic hormone and cortisone upon acquired immunity to trichinosis in mice. *Am. J. Pathol.* **30**, 913-918.

Stunkard, H. W. 1938. The morphology and life cycle of the trematode, *Himasthla quissetensis* (Miller and Northrup, 1926). *Biol. Bull.* **75**, 145-164.

Tinbergen, N. 1953. *Social Behavior in Animals.* Wiley, N.Y. 150 pp.

Virtanen, A. I., and Hietala, P. K. 1955. 2(3)-Benzoxazolinone, an antifusarium factor in rye seedlings. *Acta Chem. Scand.* **9**, 1543-1544.

Virtanen, A. I., Hietala, P. K., and Wahlroos, O. 1956. An antifungal factor in maize and wheat plants. *Swom. Kemist.* **29 B**, 143-144.

Walker, J. C. 1923. Disease resistance to onion smudge. *J. Agr. Res.* **24**, 1019-1039.

Walker, J. C. 1957. *Plant Pathology.* McGraw-Hill, N.Y. 707 pp.

Weinstein, P. P. 1953. The effect of cortisone on the development of the immune response in the white rat to *Nippostrongylus muris*. *J. Parasitol.* **39** (Suppl.), 35.

Weinstein, P. P. 1955. The effect of cortisone on the immune response of the white rat to *Nippostrongylus muris*. *Am. J. Trop. Med. Hyg.* **4**, 61-74.

Whitlock, J. H. 1949. The relationship of nutrition to the development of the trichostrongylidoses. *Cornell Vet.* **39**, 146-182.

Whitmire, C. E., and Downs, C. M. 1954. Effect of cortisone on experimental murine typhus in mice. *Science* **119**, 468-469.

HOST INFLUENCES ON SOME HAEMOSPORIDIAN PARASITES*

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THE effects of microbes on higher animals have certainly and understandably received more consideration than the converse, the effects of the vertebrate host on the parasite. Since this symposium deals with resistance and immunity in parasitic infections it seems legitimate to inquire into the effects that resistance and immunity of the host may have upon the parasites. Admittedly this general subject is too vast to be presented in a single paper or even in a single symposium. I am, however, going to look at a very narrow segment of the question—namely, the influences of the hosts, both vertebrate and mosquito, upon some malarial parasites.

Since the host constitutes the environment for the parasite we may look at the problem as a study of the environment upon the organisms serving as parasites. Now the free-living organism offers much more favorable material for such a study, and much progress has been achieved over the past forty years in analyzing the principles involved in the effect of the environment on the free-living protozoa. Moreover, among the parasitic protozoa the flagellates have served as subjects for much research with the result that the action of immune substances of the host upon the parasitic haemoflagellates has been well studied. Malarial parasites are not as favorable material for this kind of study. They are intracellular (in the vertebrate host) and are not easily removed from their host cells. Hence much of the experimentation has to be made upon the parasite-host-cell complex. Another disadvantage they offer is the fact that they do not elicit in the blood of the host, antibodies which are as susceptible to *in vitro* studies as some of the other parasitic protozoa. In spite of the disadvantages of this kind offered by malarial parasites it nevertheless is important to continue studies on the effects of their environment (that is, their hosts) upon them. It is dangerous to extrapolate from conclusions reached after the study of one group of organisms to another group and especially so when the differences between groups are as great as the differences which exist between intracellular sporozoa and extracellular flagellates.

* The opinions and statements contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

The discussion which follows is an attempt to evaluate the information available from my own published and unpublished work and from the publications of others which in any way bears upon the influence of both the mosquito host and the vertebrate host upon their malarial parasites. An effect of the host which results in the death of the parasite will be excluded from consideration since this aspect of the subject has had extensive treatment in the published literature. We shall be looking at (1) effects which are definitely brought about by residence in certain hosts but which do not persist when the parasite is transferred to another host, (2) effects of one host that persist long enough to be recognized when the parasite has been transferred to another host, and (3) more permanent effects which may be inherited through many passages through hosts.

The Effects of the Mosquito Host on Malarial Parasites

Although a discussion of the susceptibility of mosquitoes to malarial parasites might be strictly thought of as falling outside of a consideration of the effects of the host on its parasites (since one must assume susceptibility on the part of the mosquito before there can be any parasitism), nevertheless it seems appropriate to mention it, at least briefly. Mechanisms similar to those which prevent infection of the mosquito by malarial parasites may possibly produce some effect upon parasites which have succeeded in establishing themselves in the mosquito. Moreover, one may have to contend with the effects of such mechanisms when attempting to assess any possible effects of the vertebrate host exhibited by the parasite after it has been transferred to the mosquito.

It was almost 30 years ago that I found susceptibility to malaria to be a characteristic of the mosquito capable of change by experimental selection of progenies from infected and uninfected females (Huff, 1929). Subsequent studies proved that little or no correlation existed between the susceptibility of individuals of a species of mosquitoes to one species of parasite and their susceptibility to a different species. When, however, the same individual mosquitoes were fed at two different times on blood infected with the same species of parasites there was a very high correlation between the infections resulting from the two feedings (Huff, 1930). Genetic studies on *Plasmodium cathemerium* in *Culex pipiens* revealed the fact that susceptibility behaved as a simple Mendelian recessive character (1931). Many attempts, since that work was done, which have been made by breeding and selection to determine whether the degree of susceptibility of mosquitoes to malaria is hereditary have been inconclusive. However, statistical studies on the number of oocysts resulting from feedings of *Culex pipiens* on *P. cathemerium* and *P. relictum* gave indirect evidence that the degree of susceptibility of this mosquito to

each of these parasites is determined by inherent characteristics of the individual (1934).

Oocysts. The stages of malarial parasites which occur in mosquitoes are not very suitable for studying the possible effects of the host. Neither oocyst nor sporozoite continues for more than one generation. Of the two, the oocyst is the more favorable for study. Since its normal shape is spherical, simple measurement of its diameter makes possible the calculation of its surface and volume. In statistical studies on oocysts (Huff, 1940), I found that the variance of oocyst size *between* mosquitoes fed on the same infected bird, kept under similar conditions, and dissected at a given time after feeding was significantly greater than the variance *within* mosquitoes. In other words, the different environments offered by individual mosquitoes were responsible for greater variance than would occur as a result of the natural variability of the parasites within the same mosquito. As will be shown later, wide variations have been found (Huff and Marchbank, 1955) in oocyst size in mosquitoes fed on the same infected bird day after day. Also when variation due to individual mosquitoes was discounted there was some evidence that oocyst size varied when the feeding of mosquitoes was done upon different infected birds.

It has never been determined whether, when all other sources of variation are excluded, oocysts from the same species of parasite would differ significantly in size when grown in different species of mosquitoes. I have some unpublished data on the size of oocysts of *P. cathemerium* in *Culex pipiens* and *C. tarsalis.* In two instances the oocysts in *C. tarsalis* are significantly larger than those in *C. pipiens* while in a third they are not. The amount of work involved in determining this point would appear to be out of all proportion to the significance of the finding. It would certainly appear to be expected that species differences in mosquitoes would be a contributing factor toward variation in oocysts, since the differences in individual mosquitoes of the same species are so strongly reflected in the variations among the oocysts growing in them.

Regarding the nature of the real causes of variation in oocyst size we know very little. I have previously shown (1941a) that oocyst size is not influenced by (1) the degree of infection in the individual mosquito, (2) by the activity or age of the mosquito, or by (3) the humidity of the environment of the mosquito. The variability in size of oocysts in a clon was less than that in the parent strain but when the clon was passed through a mosquito, thus undergoing sexual reproduction, the larger variability of the parent strain was not restored (1941b). This is in sharp contrast to similar studies made by Taliaferro (1926) on *Trypanosoma lewisi.* A clon of this trypanosome showed no differences in variability

within different rats even when passed through a long series of subinoculations. However, after this clon had undergone a passage through the flea, *Xenopsylla cheopis,* the variability in its total length had increased. One is tempted to question whether a malarial parasite is protected more by its intracellular position than a trypanosome which is exposed to the changes which occur in its habitat, the blood plasma of its host.

Although one can conjecture that all of these effects produced on the malarial oocysts are the results of changes in the immediate environment of the parasite, that is, the biochemical conditions in the host cell, we must admit our lack of direct knowledge on this subject.

Sporozoites. The sporozoite stage does not offer the same opportunity for quantitative measurement as oocysts do. It is, at present, impossible to determine accurately the numbers of sporozoites produced in a given mosquito and it is very difficult, if not impossible, to determine the numbers which reach the salivary glands of the mosquito. Barber (1936) could find no single factor which seemed to be responsible for the degeneration of sporozoites in the salivary glands of mosquitoes. It was not associated with humidity nor with the food of the mosquitoes. He did not believe that age or low temperatures were necessarily factors in causing degeneration of sporozoites in any of the species which he studied. He did, however, find a greater degree of degeneration of sporozoites in *Anopheles superpictus* than in *A. elutus* thus proving the very effect of species of host on the sporozoite which we have already noted would be difficult to prove for oocysts.

The Effects of the Vertebrate Host on Malarial Parasites

Morphological changes. Differences in the morphology of the parasite and of the infected erythrocytes have been noted in several species of malarial parasites when observed in different species of hosts. Knowles and Das Gupta (1932) studied the morphology of a malarial parasite from the monkey, *Cercopithecus pygerythrus* (which was later named *Plasmodium knowlesi* by Sinton and Mulligan, 1932) experimentally established in seven different species of primates including man. In the host from which it was isolated they found this parasite resembled morphologically *P. vivax* in man; the infected erythrocytes were pale and enlarged, they contained distinct Schüffner's dots, and the growing trophozoites were very ameboid. In *Macacus rhesus* the infected erythrocytes were not enlarged and they exhibited no stippling. In many respects it resembled *P. falciparum* in man. When observed in human infections there was little or no ameboid activity of the trophozoites, the erythrocytes were not enlarged, and there was no stippling of the infected cell; in some respects it resembled *P. malariae* in man.

Similarly, the Taliaferros (1934b) observed differences in the morphology of *Plasmodium brasilianum* in various Panamanian monkeys. This species of parasite was studied either in natural or experimental infections in black and red spider monkeys, black and brown howlers, the night monkey, the white-throated monkey and the marmoset. Among the differences observed in the parasites in the different primates studied were: slight differences in the size of the infected red cells, the frequence of occurrence of band forms and the numbers of nuclei in the schizonts. The most spectacular difference observed was in the preponderance of band forms in black and brown howlers (*Alouatta palliata inconsonans* and *A. p. palliata*) and to a lesser degree in the night monkey (*Aotus zonalis)* as contrasted to other species such as the white throat *(Cebus capucinus).* On the other hand the Taliaferros (1934a) observed no morphological differences in the asexual stages of the human parasite, *P. falciparum,* after it was transferred to the howler monkey.

Wolfson (1939) observed that the gametocytes of a matutinal strain of *P. relictum* which are round in the canary became elongated when produced in domestic ducks. Manwell (1943) noted that *P. nucleophilum* and *P. relictum matutinum* were altered more by residence in chickens than they were in ducks. *P. nucleophilum,* which received its name because of its affinity for the nucleus of the host cell when grown in the canary, was seldom in contact with the nucleus when grown in the chicken.

In addition to effects of the host on the erythrocytic parasites which have been recorded, some effects on the exoerythrocytic stages have been found. Many interesting differences were observed in the morphology of the pre-erythrocytic stages of *P. lophurae* in different species of hosts (Huff, Coulston, Laird, Porter, 1947). Some of these obviously represented degenerative changes; others appeared to be less severe. The effects of residence in some hosts such as the duck appeared to be more harmful than in others (guinea fowl, chicken). In addition, some of the morphological abnormalities observed in the parasites in turkeys might possibly have been due to slight interference with the normal processes of schizogony. Similar abnormalities to those observed in *P. lophurae* have also been seen in pre-erythrocytic forms of both *P. relictum* and *P. gallinaceum* in ducks (Huff, 1951).

When these morphological changes are studied carefully the conclusion seems justified that they are of very little significance as evidence that any real change has been brought about by the host. Such criteria as enlargement or stippling of the host cell are actually changes brought about by the parasite upon the host cell. The particular form or position in the cell assumed by the parasite may be the result of slight physical or

chemical differences in the host cell and differs in no manner or degree from the changes in behavior exhibited by all animals when placed in different environments. Since most of the observations on changes of the parasites resulting from residence in different species of hosts were made on fixed and stained preparations it may be that many of the differences observed do not even represent behavior differences but may be brought about by different reactions of the host cells to the fixing and staining reagents.

If wide differences in host cells were to be expected to produce real morphological changes in the parasites one might expect that they would be spectacularly in evidence in the experiments of McGhee (1951). He was able to adapt the avian parasite, *Plasmodium lophurae,* to continuous passage in infant mice. Although slight alterations in morphology of this parasite were observed in its first few passages in mice it very quickly tended to approach the morphology which it exhibited while parasitizing the cells of the bird.

We may conclude, therefore, that differences in morphology observed in parasites in different species of host reflect nothing more than transient expressions of reaction to changed environments.

Merozoite number. Beginning with the work of Boyd and Allen (1934) various workers have found that the mean numbers of merozoites in the mature schizont fall significantly during the crisis in parasitemia. The Taliaferros found this to occur in infections of monkeys with *P. brasilianum, P. cynomologi* and *P. knowlesi* (1944, 1947, 1949); and Thompson (1944) described similar findings for *P. floridense* in lizards. This effect of the host on the parasite is very interesting since it is susceptible to statistical treatment. The cause of the decrease in number of merozoites produced by a schizont has been attributed by the authors mentioned to the development of immunity by the host. We have failed to find any decrease in merozoite number either in *P. cathemerium* (Huff and Marchbank, 1955) or *P. gallinaceum* (Huff, Marchbank, and Shiroishi, 1958) infections. In the latter case we were attempting to determine the effects of various additives to the blood and diet of the hosts upon infectiousness of gametocytes. Merozoite counts on *P. gallinaceum* which received supplement of calcium pantothenate in their diet were significantly higher than counts made on birds which had not received this supplement. In an earlier paper, Brackett, Waletzky, and Baker (1946), found the merozoite counts were lowered in the schizonts of *P. gallinaceum* in chickens fed upon diets deficient in pantothenic acid as compared with the counts in birds on diets supplemented by pantothenic acid. Because of the discrepancy in the results reported by these two groups of workers more work is needed to establish whether the cause of the decrease in mero-

zoite numbers is the result of immunity or of a developing nutritive deficiency in the animal during the course of infection. Whatever the explanation turns out to be it still remains as one of the most interesting effects of the host on the parasite. It is, however, a temporary effect which is not transmitted to subsequent generations of parasites in other individual hosts.

Strain differences. I shall discuss next certain differences in strains of parasites which have been observed during experimentation upon them and their hosts. It will be apparent from this discussion that it is questionable whether one can justify including this section under Effects of the Vertebrate Host on the Parasite. Regardless of where it is placed, however, I feel that it does belong, at least indirectly, in any treatise of this kind.

Haas and co-workers found that certain chicks which had been inoculated with sporozoites of *P. gallinaceum* developed fatal infections and were found at autopsy to have exoerythrocytic stages in their brains (Hass, *et al.*, 1946). Death resulted either before erythrocytic parasites had made their appearance or (in the opinion of these authors) before there were sufficient numbers of exoerythrocytic stages to account for fatality. They observed that infections of this type resulted in 90 per cent of chicks infected by mosquitos infected from a strain passed alternately from mosquito to chick.

Subsequently they (Haas, *et al.*, 1948) reported that these infections characterized by heavy exoerythrocytic infection early, which caused the death of the chick before erythrocytic development appeared, could result from a number of different experimental treatments. These were: (a) inoculation of chicks with emulsions of brains containing exoerythrocytic stages, (b) similar inoculations into embryos, (c) occasionally in embryos infected by mosquitoes infected from a strain alternately passed from mosquito to chick, (d) inoculation into chicks of sporozoites derived from parasites of a blood-inoculated strain, and (e) by suppression of the erythrocytic forms by quinine. They remarked upon the regularity of occurrence of such infections under the artificial treatment in the laboratory and the rapidity with which the patterns thus produced could be changed. Lewert (1950a) found that similiar infections resulted in chicks inoculated with tissue cultures containing only erythrocytic forms. Lewert (1950b) also found that such strains maintained their characteristics through 26 serial passages in young chicks and that in some of the animals unpigmented erythrocytic parasites appeared in the blood. Serial passage of blood starting from birds dying of the exoerythrocytic strain resulted in reversion to the more normal type of infection which results in the usual serial transfer by blood of the unaltered strain. Lewert postu-

lated that such strains resulted from separation of one stage of the parasite from another through survival in tissue culture of parasites with the potentiality of living in fixed tissue cells whereas in strains passed regularly by blood inoculations there is a selection in favor of parasites which are prone to enter red cells.

Although the strain differences which were described in the publications just cited would appear to be mainly the result of differential selection effects of various experimental procedures on populations of parasites (usually referred to as "strains") the longer-term experiments of Greenberg, and co-workers indicate that more permanent alterations may occur in strains (Greenberg, Trembley, and Coatney, 1950, '51; Trembley, Greenberg and Coatney, 1951a, b; Greenberg, 1955). They observed the appearance and behavior of two strains (BI and M) differing markedly from each other and from the parent strain (SP) in pathogenicity, in the production of exoerythrocytic and of erythrocytic stages. These strains retained their distinguishing characteristics for 6 to 12 years even when transmitted by means of the techniques believed to be responsible for changes in strains previously. Although this work is extremely important and interesting it is hardly pertinent here, for it is difficult to assess the relative importance of mutation of the parasite and effect of the host. In fact it would appear from careful consideration of all of their findings that the effect of the host may be only an accidental one in which, by virtue of the obligate alternation of vertebrate with invertebrate host, the host acts as a screening agent to remove the mutations which may be perpetuated in the artificial methods of transfer in the laboratory.

Host cell selectivity by the parasite. As long as only the erythrocytic stages of malarial parasites were known one of the most interesting characteristics of the parasite – namely its preference for certain types of host cells – was unknown. With the discovery of exoerythrocytic stages it was soon shown that (1) the species of host inhabited was important in determining the types of cells selected by the parasites and (2) that during this residence in a particular host the parasite underwent notable changes in its preference for different types of cells of this host.

Wolfson (1940) found that a strain of *P. cathemerium* isolated from a wood-thrush produced exoerythrocytic stages when maintained in canaries but failed to produce them when transferred to ducks. Likewise Rodhain (1938a, b) found that a strain of *Plasmodium relictum* found in penguins produced abundant exoerythrocytic stages whereas when it was transferred to canaries no exoerythrocytic stages could be found.

Thompson and Huff (1944) studied the distribution among various blood cells of the asexual forms of *Plasmodium mexicanum* while living in five different species of lizards. The predominant cell types infected in

Sceloporus undulatus and *S. olivaceous* were erythrocytes, normoblasts, and erythroblasts. In *Crotaphytus collaris* and *Phrynosoma cornutum* the cells most preferred were lymphocytes and monocytes, whereas thrombocytes were preferred above all others in *Phrynosoma asio*. It should be emphasized that all blood cell types were parasitized in all five species of these lizards. The great differences were in the distribution of the parasites among the different cell types. In the observations just described we were dealing with a species of parasite with a wide range of ability to live in various types of cells of the host including all of the blood cells, the blood-forming cells, the cells of the lymphoid-macrophage system, and the true endothelial cells. In species the exoerythrocytic stages of which belong to the gallinaceum-type, the kinds of cells are narrowed down to the lymphoid-macrophage system and endothelial cells. Consequently in observations made on the cell preferences of these parasites in various hosts it may only be known that exoerythrocytic stages are or are not present. A comprehensive study was made upon the presence or absence of erythrocytic and exoerythrocytic stages of *P. relictum* in several birds of the family *Columbidae* (Huff, 1948). In some of the birds inoculated by infected blood and in some inoculated with sporozoites parasitemia was established but no exoerythrocytic stages could be demonstrated. Many instances of this kind are on record in which parasitemia may be produced without any indication that exoerythrocytic infection may occur. Moreover, pre-erythrocytic stages of *P. gallinaceum* have been found in the canary, a host in which no parasitemia with this parasite has yet been produced either by sporozoite or trophozoite inoculation (Huff, 1951). One may question, however, whether these examples represent true inabilities on the part of the parasites to live in particular cell types or whether, as in the case of *P. mexicanum* which we have just discussed, the differences are relative. In instances where tissue stages have not been described in animals exhibiting frank parasitemia the parasites may have been so difficult to detect that they were not found. In the example just cited of a parasite capable of producing pre-erythrocytic stages but no erythrocytic stages, we may likewise ask whether the numbers entering erythrocytes were small initially and were removed from the blood by the immune mechanisms faster than they could multiply.

Changes during the course of infection in the vertebrate. Interesting as are these differences in host cell preferences in different species of host the changes which take place in the selectivity of host cells by the parasite during the course of infection in an individual host are of more interest to us here; for there would seem to be hope of analyzing the changes occurring during the course of infection in an individual which

affect this characteristic of the parasite more readily than of determining the differences between host species which bring about similar differences in the preferences shown by the parasite for the various types of host cells.

In infections produced by sporozoites of avian malaria it has been established (at least in certain species) that a few generations of pre-erythrocytic stages live in cells of the lymphoid-macrophage system followed by a shift to the endothelial cells of veins and capillaries (Porter, 1942; Huff and Coulston, 1944). Erythrocytes are invaded concomitant with or subsequent to the invasion of the endothelial cells. In blood-induced infections (of the gallinaceum-type) rapid development of parasitemia occurs for several days followed by invasion of the reticular cells of the Schweigger-Seidel sheaths of the spleen. Endothelial cell invasion occurs last and, in infections such as *P. fallax* and *P. lophurae* in turkeys, brings about death of the animal after it has survived the parasitemia (Huff, 1954, 1957). I should like to point out that the endothelial cells of the veins and capillaries are so situated that they must be passed over a great many times by the various stages of the parasite which circulate in the blood. In spite of their strategic location they are usually the last to be invaded. However, when their invasion does take place it is massive and apparently unresisted.

Many unpublished experiments have been carried out in my laboratory aimed at determining, first, whether this spectacular change comes about as the result of some change in the host which causes certain cells to change their susceptibility to attack by the parasites; whether during the course of the infection the parasite somehow changes in its ability to select these cells; or whether the changes come about as the result of some influence affecting both the susceptibility of the host cells and the ability of the parasite to approach and invade these cells; and second, aimed at determining what mechanisms bring about this change. As yet, there is no definite answer to either question. At least three things can be thought of as resulting in a host during its infection with malarial parasites: (1) it may be depleted of some of its normal substances; (2) it may develop immunity to the malarial antigens; and (3) some malfunction in one or more organs may develop. My experiments have been aimed at altering the usual relations in such ways that the changes which are observed to occur in the host cell selectivities by the parasite can be explained on one or more of these hypotheses. The hypothesis that some inherent and spontaneous change such as mutation may occur in the parasite is discarded because of the close correlation between the changes observed in the parasites and the time that has elapsed since the host acquired its infection. I must leave you in suspense regarding the outcome of this mystery story for at this point it cannot be written.

Tissue culture. For the past three years we have been studying exoerythrocytic stages of *P. gallinaceum* and *P. fallax* in cultures of chick embryo tissues in our laboratory. On the basis of this experience and from publications of others I am going to be bold enough to suggest that the differences between the behavior of malarial parasites *in vivo* and *in vitro* are comparable to the differences observed in their behavior in two species of host. I am not suggesting that tissue cultures be described as a new species of host! However, I believe it is helpful in thinking about the differences in behavior in the parasites to think of tissue culture as another kind of host. We are hopeful that the use of this new "host" will make possible some progress in analyzing the mechanisms which effect changes in the selectivity of cell types by the parasite. Dubin (1954) grew *P. gallinaceum* in cultures of chick liver and lung and after these cultures were fixed and stained he found all of the stages of exoerythrocytic schizogony in hepatic and pulmonary epithelium. We are inclined to believe on the basis of our observations made by the phase contrast microscope on similar preparations that the hepatic epithelial cells are invaded by the parasites soon after the cultures have been made. These observations and those of Dubin are entirely different from those made on the tissues of infected animals by my co-workers and me, for in no instances did we ever observe in the many thousands of preparations studied of avian and saurian tissues any evidence that hepatic cells of epithelial origin were invaded. We look toward the establishment of clons of host cells as a step in the direction of the solution of the fundamental changes which occur before the susceptibility of the host cell to the parasite is changed so spectacularly.

Primate malaria. Although the cell types for a number of species of simian and human malarial parasites have been identified by Garnham, Shortt and their co-workers and several other investigators, I consider that these findings need to be put on a firmer basis before one would be warranted in making the kind of conclusions that I have made on the findings of avian and saurian malaria.

Effect of the vertebrate host on the gametocyte. Of all effects produced on the parasite by the host the qualitative and quantitative changes in gametocytes are some of the most interesting and most amenable to study. Change in numbers (including total loss) of gametocytes is easily determined from studies of the blood of the host and changes in ability to initiate the sporogonous cycle can easily be tested by dissection of mosquitoes and determination of the presence and number of oocysts produced in them. The various effects known can be classified as to whether they result (1) from differences in species of host, or (2) from various changes within the species or individual hosts.

The effects of different species of hosts on the presence of gametocytes

in the blood were clearly shown by Thompson and Huff (1944) in their studies of *Plasmodium mexicanum* in various kinds of lizards. This species produced gametocytes in *Sceloporus ferrariperezi,* the Mexican lizard from which it was isolated. However, it was passed serially several times in the collared lizard (*Crotaphytus collaris*) without the appearance in the blood of any gametocytes even though asexual stages were abundant. After 4 or 5 passages in the collared lizard without the appearance of gametocytes it was passed to two different species from the same genus as the species from which it was originally isolated, namely, *S. undulatus* and *S. olivaceous.* No gametocytes were produced when transfers were made from these two species into *Phrynosoma asio* and in only two individuals out of eleven *Phrynosoma cornutum* inoculated from lizards in which gametocytes were produced. It is interesting to note that in the collared lizard in which no gametocytes were observed the infection became predominantly exoerythrocytic although it is unknown whether these two characteristics exhibited by the parasite in this host were in any way related.

Another example of the inability of a malarial parasite to produce gametocytes in an abnormal host was given by Taliaferro and Taliaferro (1934a). They succeeded in establishing blood infections of the human malarial parasite (*P. falciparum*) in howler monkeys (*Alouatta sp.*) but were unable to demonstrate any gametocytes in any of the monkeys infected. More recently, Bray (1958) has had similar results from inoculating *P. falciparum* into chimpanzees.

Wide differences have been observed in the number of gametocytes produced by *P. fallax* in various birds in our laboratory (Huff and Marchbank, 1955, and hitherto unpublished data). These numbers are low in chickens, goslings, and guinea fowl, regularly high in turkey poults and very irregular in pigeons. As we have previously shown (Huff and Marchbank, 1955) the differences in numbers of gametocytes produced in different individual pigeons are as great as any differences in the numbers produced in different species of birds. However, there was great regularity in the numbers produced in individual turkey poults. One is tempted to inquire whether the explanation for such differences in species of host may be due to the relative degrees of genetic homozygosity in the stocks used; the turkey poults were of the highly inbred Beltsville line whereas the pigeons were from a wide variety of sources.

Similarly, wide differences in the degree of infectivity of gametocytes for mosquitoes is exihbited by *P. fallax* when grown in different species of birds. Gametocytes produced in turkey poults were regularly better infectors of mosquitoes than those produced in chickens. One of the most spectacular examples of these differences was recorded (Huff and March-

bank, 1955) in the infectivity of gametocytes produced in guinea fowl as compared with those produced in chickens. In spite of the fact that gametocytes were so low in number in guinea fowl as to escape being found on microscopic examination on 5 out of 8 days, oocysts were produced in mosquitoes in numbers greater than those produced from much greater numbers of gametocytes in chickens. Qualitatively the gametocytes produced in the two species of birds were vastly different in ability to infect mosquitoes. Another striking example of this type of effect of the vertebrate host on the gametocyte is represented by the observations made on the 1 P strain of *P. relictum* isolated by Coatney (1940) from a pigeon. Numerous attempts were made by various investigators, between the years 1938 and 1944, to infect several species of mosquitoes (*Culex pipiens, C. quinquefasciatus, Aedes aegypti, A. albopictus, Anopheles quadrimaculatus* and *A. crucians*) by feeding them on the blood of pigeons infected with this strain. These attempts were unsuccessful. However, Redmond (1943, 1944) found that *Culex pipiens* could be readily infected by this strain after it had been adapted to canaries. We have since shown (unpublished data) that the infecting power of gametocytes of the 1 P strain produced in pigeons is not completely absent but can be shown to exist when *Culex tarsalis* is used as the test mosquito. The effect of the pigeon in reducing the infectability of the gametocytes is, nevertheless, as interesting as would be its ability to completely sterilize them. In fact the same mechanisms are probably involved regardless of whether the infectability is completely destroyed or only greatly reduced. This question will receive further elaboration in a paper now in preparation.

Many of the effects produced on gametocytes by different species of vertebrate hosts are found to occur in the individual hosts of the same species and, in addition, some very interesting effects can be followed in a single vertebrate host during the course of the infection which it undergoes. I have already called attention to the great variations in the numbers of gametocytes which appear in the blood of different individual infected birds. This is a common observation in other types of malarial infection, including human malaria. No progress has yet been made in working out the explanation of these large differences exhibited by the parasites in different individuals.

Some very interesting examples have been described of the loss of the ability to produce gametocytes both in avian and in human malaria. The first record of such a strain was made by Barzilai-Vivaldi and Kauders in 1924 who were working in Professor Wagner-Jauregg's clinic in Vienna. Cuboni (1926) reported that two other strains obtained from the same clinic did not produce gametocytes after undergoing 134 and 143 pas-

sages in patients. Similar observations have been made by Plehn (1925) in Palestine, and Kopeloff (1930) in New York. Boyd (1945) reported loss of gametocytes in a strain of *P. falciparum* from Trinidad and Jeffery (1951) described the occurrence of a similar strain of this species isolated in a patient from South Carolina.

In 1932, while I was at the University of Chicago, a strain of *P. cathemerium* in canaries which had been producing gametocytes in large numbers ceased to produce them and continued to be unable to do so for as long as the strain was kept – a period of well over 10 years (Huff and Gambrell, 1934). All experimental attempts to induce the production of gametocytes by this strain failed. The studies which Miss Gambrell and I made on this strain and those which Miss Gambrell (1937) subsequently made showed rather conclusively that the ability to produce gametocytes by a strain was capable of change but that, in general, strains tended to produce a certain level of gametocytes through many blood passages. Whether or not these changes came about as a result of some effect of the host upon the parasites was never clearly settled. However, Caldwell (1944) noted that there was a concomitant loss in periodicity of asexual reproduction in a strain which was losing its gametocytes as a result of experimental exposure to a temperature of 50°C for 8 minutes. The strain subsequently lost its ability to produce gametocytes and its synchronism of asexual reproduction. Although these changes were presumably due to heating of the parasites *in vitro* it seems likely that the host could effect similar changes. During the period when we were observing the spontaneous appearance of gametocyteless strains in the laboratory we noted that they arose during epizootics of fowl pox in the canaries. Whether the parasites were affected by the virus of this disease was never proved but it is an interesting speculation. These gametocyteless strains were apparently the result of some effect different from the temporary loss of gametoctyes which were observed in lizards of different species.

Relapse. The occurrence of relapse in human and animal malarias is a subject upon which there is a voluminous literature. Any change in the behavior of the parasite as marked as relapse should be examined with the query in mind as to whether this change is an effect produced by the host and, if so, what mechanism or mechanisms are involved. I believe I am correct in saying that the preponderant evidence favors the belief that relapse is due to some change in the host. Furthermore, the most generally accepted view is that changes in the immunity of the host are the most likely cause of relapse. It would enlarge the scope of this discussion too much to attempt to review the significant literature dealing with the relationship between immunity in the host and relapse. However, one

other effect which may possibly fall within our arbitrarily chosen limits is the effect of hormonal changes upon relapse.

O'Roke (1934) reported that in ducks which had survived an infection with *Leucocytozoon simondi* there was a subsequent decrease in parasitemia until the middle of the winter when the blood became free from parasites. He found that parasites reappeared in the blood in the spring and he considered that this represented natural relapse of the infection. In the spring of 1941 I studied the blood of 28 semi-domesticated mallard ducks at Land O'Lakes, Wisconsin, at approximately monthly intervals and was able to confirm O'Roke's finding on the reappearance of the parasites in the blood of ducks in the springtime (Huff, 1942). In the paper reporting these findings I said in reference to this phenomenon of relapse, "It would be interesting to determine whether or not this phenomenon is in any way correlated with the physiological ossification of bone marrow antecedent to egg laying, with the consequent removal of myelocytopoietic cells from the tissue." Chernin (1952) ten years later was able to establish such a relationship between relapse of *Leucocytozoon* infections in ducks and the onset of reproductive activity in the host. He reported that ducks suffered relapses beginning in February and March. His animals were kept in the laboratory and, hence, the appearance of new infections was ruled out. Moreover, he was able to precipitate egg-laying in ducks which carried latent infections several weeks to several months earlier by increasing the amount of time they were subjected to artificial light during the fall and winter months. There was a corresponding shift in the time of occurrence of the relapse; indicating that there is, in fact, some relationship between the hormones connected with reproduction in the host with the release of parasites to produce a relapse. We must not rule out, of course, the possibility that the action of hormones may not be a direct one but may bring their effect about indirectly by altering the state of acquired immunity in the host. I have introduced this subject under the heading, The Effect of the Host on the Gametocyte, because the parasitemia in *Leucocytozoon* consists entirely of gametocytes. However, the production of gametocytes must involve the activity of shizogonous forms from which the young gametocytes develop and, therefore, the effect of the host may possibly be upon the exoerythrocytic or tissue stages.

Effects produced during the course of infection. We have, for several years, been attempting to analyze the very interesting effect noted by Lumsden and Bertram (1940). They reported qualitative changes of the gametocytes of *P. gallinaceum* during the course of infection in chickens. This effect was exhibited as a decreasing ability of gametocytes to produce infections in the mosquito, *Aedes aegypti,* as the infection in the

chicken progressed. Cantrell and Jordan (1946) and Eyles (1951, 1952a, b) made further studies on this characteristic of *P. gallinaceum* and each offered hypotheses to explain the phenomenon. In our laboratory we extended the study to seven host-parasite combinations involving the parasites, *P. gallinaceum, P. fallax,* and *P. cathemerium;* the avian hosts, chicks, pigeons, guinea fowl, turkeys and canaries; and the mosquitoes, *Aedes aegypti, A. albopictus, Culex pipiens,* and *C. tarsalis* (Huff and Marchbank, 1955). Without exception we found the same pattern of change in gametocyte infectiousness in these combinations of host and parasite as had been found for *P. gallinaceum* in the chicken by the authors just cited. In general, the typical result was like that shown in figure 1 (Fig. 3, Huff and Marchbank, 1955). There was an early rise in the mean number of oocysts produced in the mosquitoes followed by a fairly sharp and continuous fall, and the outstanding fact was that this fall in numbers of oocysts began at a time when the numbers of gameto-

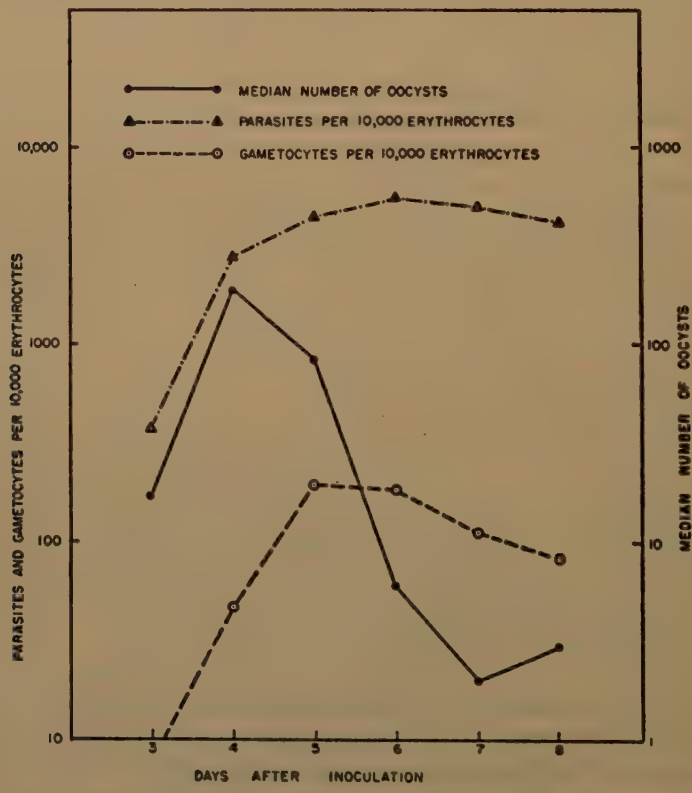

FIG. 1 (From Huff and Marchbank, 1955; fig. 3). Oocysts of *P. cathemerium* produced in *Culex pipiens* mosquitoes fed on a canary daily during the course of its infection.

cytes in the blood of the bird at the time that the mosquitos took their blood meals was rising. That this indicated a real change in the quality of the gametocytes is shown even more clearly in figure 2 (Fig. 7, Huff and Marchbank, 1955). In this experiment two species of mosquitoes (*Culex pipiens* and *C. tarsalis*) were fed daily on each of two canaries infected with *P. cathemerium*. The mean numbers of oocysts resulting in the two species of mosquitoes are indicated by the solid lines in the figure. Al-

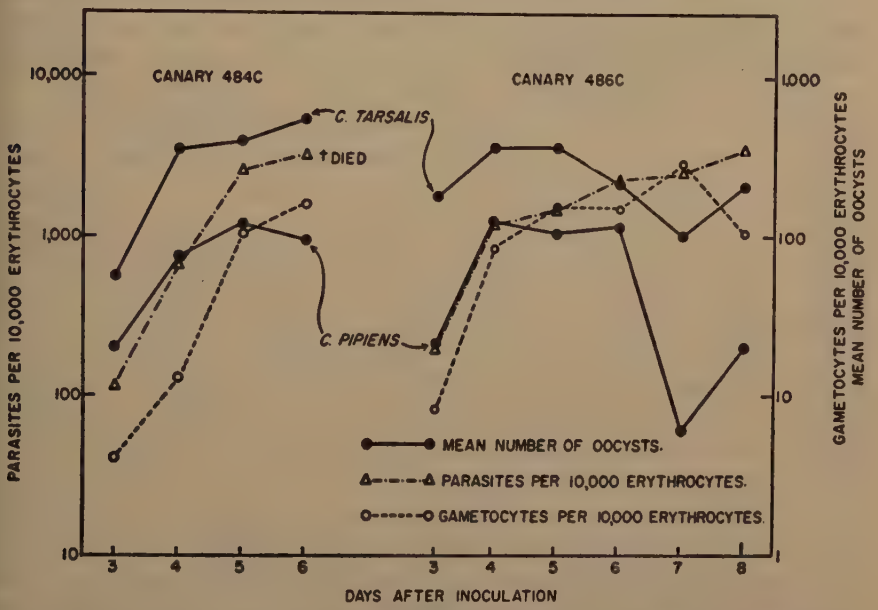

FIG. 2 (from Huff and Marchbank, 1955; fig. 7). The patterns of oocysts of *P. cathemerium* in two species of *Culex* fed daily on each of two canaries during the course of their infections.

though at all times the number of oocysts produced in *C. tarsalis* were higher than those in *C. pipiens* there is a very close parallelism between the graphs for the oocysts in the two kinds of mosquitoes. This even holds for the slight upturn in infectiousness of the gametocytes in canary 486C on the 7th day of the infection – a characteristic of this particular species combination.

A very extensive series of studies has been carried out attempting to determine the factors which bring about such changes in the gametocyte. The results will be published in detail in the near future (Huff, Marchbank, and Shiroishi, 1958). Two hypotheses to account for the changes were tested; namely (1) that they were the result of deficiencies in the host resulting from the infection of the bird, and (2) that they were the

result of the development of an active immunity. The first hypothesis was tested in experiments of two kinds: (a) the administration of substances assumed to be depleted from the blood of birds as the infection progressed and (b) the experimental acceleration of a possible depleting action by bleeding the host during the infection. The substances injected at daily or half-daily intervals were: uninfected whole blood, coenzyme A, ferrous sulfate, sodium glutathione, calcium pantothenate, and sucrose. There was no evidence either that the substances supplied or the more rapid depletion from bleeding influenced the pattern of gametocyte infectiousness. Some of these results are expressed in figures 3a and b.

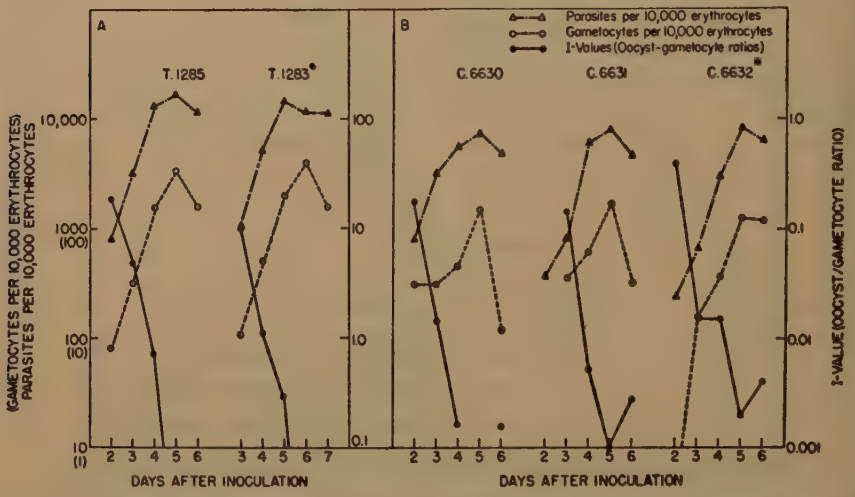

Fig. 3 (from Huff, Marchbank, and Shiroishi, 1958, fig. 1). A. Infectiousness of gametocytes of *P. fallax* for *Aedes alboptictus* in turkeys. Number 1285 (left) received 3 daily intravenous injections of 5 ml. of normal blood (days 4 through 6), and 1283 (right) received none. B. Infectiousness of gametocytes of *P. gallinaceum* for *Aedes aegypti* in chickens. Numbers 6630 and 6631 received twice daily doses of 6 ml. of sucrose *per os* (beginning on day 1) and control 6632 received none.

In explaining these figures it should be noted that we adopted a more direct way of indicating the differences in ability of the gametocyte to produce oocysts in the mosquito. The solid line here represents a value, *I*, which is the quotient of the numbers of oocysts produced divided by the number of gametocytes in the blood of the bird at the time of feeding. It will be seen that this value is independent of the actual numbers involved. When graphed in this manner it becomes clear that the gametocyte infectiousness, *I*, usually drops in value from the beginning of the test and that the gametocytes are most infectious for mosquitoes as soon as they appear in the bird. In these figures the treated animals are on the left; the controls on the right. In figure 3a three daily doses of 5 ml. of

normal blood were given intravenously on days 4, 5, and 6; in 3b, birds C6630 and C6634 received 6 ml. of 20% sucrose twice daily throughout the infection, C6632 received none. In figure 3a the infections were by *P. fallax* in turkeys and the mosquitoes used were *Aedes albopictus;* those in 3b were by *P. gallinaceum* in chickens tested by *Aedes aegypti.* It is apparent that in these experiments no real differences were shown in the patterns of change in gametocyte infectiousness in the treated animals as compared with the controls. These figures are typical of the results obtained in 14 experiments involving the administration of supplements and in 2 experiments in which from 5 to 31 ml. of blood were taken from the experimental animals for 3 days in one experiment and for 5 in the other.

In testing the hypothesis that the state of acquired immunity might have an effect on the infectiousness of the gametocytes, several types of experiments were performed. These were (1) transfusion of infected blood into a naturally immune host, (2) passive transfer of serum from hyperimmunized birds, (3) transfusion of infected blood to birds with acquired immunity and (4) active immunization of birds with infections of killed parasites prior to inoculation of live ones. The experiment on transfusion of blood infected with *P. gallinaceum* into a duck indicated a rise in infectiousness of the gametocytes when transferred to the duck. No indication of effect of passive transfer of immunity was observed. In two experiments on transfusion of infected blood into birds with acquired immunity no effect was observed in one; in the other there was a rapid fall of infectiousness of gametocytes. All four experiments testing the effect of active immunization were consistent in showing a deleterious effect on the infectiousness of the gametocytes of active immunization. The results of all of these experiments can better be explained on the hypothesis that active immunity is one of the major causes, if not the only cause, of the observed decline in the ability of gametocytes to infect mosquitoes. Figure 4 depicts the results of one of the experiments on active immunization of two chickens treated with killed parasites (C7008, C7009), and of one which received killed uninfected erythrocytes (C7013), in each instance the inoculations being given for 3 days prior to the infecting dose of parasites. The control chicken (C7015) received no inoculations other than the infecting blood. The mean values for the four birds receiving killed parasites and for the two controls are depicted on the right of fig. 4. These results are significant in indicating an earlier decline of infectivity values in animals receiving killed parasites. Since active immunity begins to appear early even in the controls because of the developing infections, one could not expect that any difference would be demonstrable between experimental animals and controls except in the very early part of the infection.

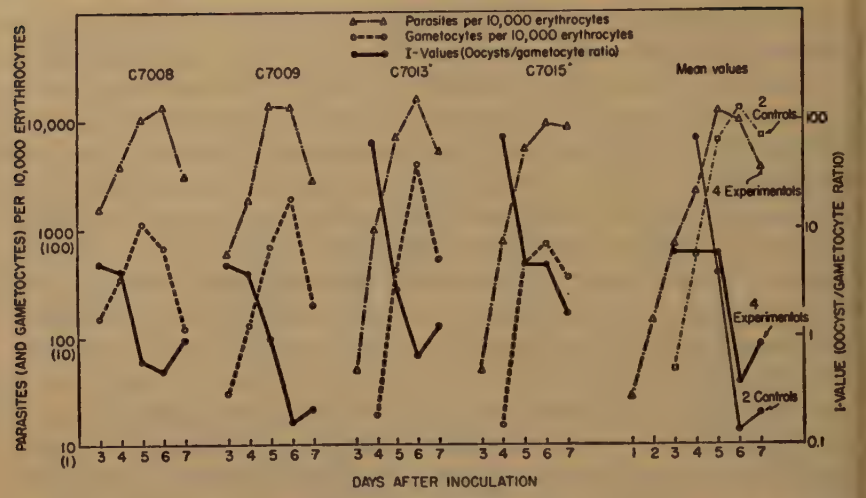

FIG. 4 (from Huff, Marchbank, and Shiroishi, 1958, fig. 5). Infectivity of gametocytes of *P. gallinaceum* for *Aedes aegypti* in two chickens (7008, 7009) which received daily intravenous inoculations of 3.5 ml. of killed parasites for 3 days prior to the inoculation of live parasites. Control, 7013, received similar doses of killed normal turkey erythrocytes and control, 7015, received no inoculations other than the infecting blood. The mean *I*-values (oocyst-gametocyte ratios) for 4 experimental and 2 control birds are also portrayed.

One must assume that the observed decrease in infectiousness of the gametocyte constitutes a very sensitive test of the production of immunity in the host by the antigens of the parasites since the effect could be observed before any appreciable effect could be observed upon the asexual stages. It must be a humoral immunity since its effect is observed after the gametocytes have been removed from the host in which cellular mechanisms are in operation. It is well known that the death of many parasites — both sexual and asexual — comes about as the result of immunity of the host. Is the effect on the ability of the gametocyte to infect the mosquito due to the same humoral mechanism which, perhaps, combined with the cellular mechanisms of the host brings about the death of the parasites in the vertebrate host? We do not know the answer, but we can only say that the end result on the individual parasite is the same since, although in the case of the gametocyte the immediate effect is not death, the gametocyte is prevented from reproducing. In spite of this spectacular action we must admit that usually this action is not effective in preventing malarial transmissions by mosquitoes for the reason that all gametocytes are usually not so affected and if a single oocyst is produced in the stomach of a mosquito, that mosquito may be capable of transmitting malaria.

Discussion and Summary

We have now passed in review a great variety of effects of both the invertebrate and vertebrate hosts upon their malarial parasites. Some of these occur under natural conditions but the majority of them have been brought into evidence through experimental studies in the laboratory. The actual mechanisms by which the host produces these effects are in most cases not well known. In some instances it is not clear as to whether the change which is observed in the parasites is caused by the host or whether other factors are responsible.

More of the effects which we have discussed here fall into the group of effects which do not persist when the parasites are transferred to another host than into other categories. They include: (1) the effects of different individuals and different species of mosquitoes upon size of the oocysts; (2) the morphological changes in erythrocytic and exoerythrocytic stages by the vertebrate host; (3) the possible effects of the vertebrate host on the number of merozoites per schizont; (4) the effects of different hosts (and of tissue culture) upon the type of host cell which is invaded; (5) the effect of changes in the vertebrate host during the progress of the infection upon the kinds of host cells invaded; (6) the effects of different species of host and of different individual hosts upon numbers of the gametocytes produced; and (7) the effects of physiological changes in the hosts upon the numbers of gametocytes produced in *Leucocytozoön* infections. One can be reasonably sure that these changes are all evanescent ones and play negligible, if any, effects upon the behavior of the parasites in subsequent passages through other hosts.

In the next category we may place those effects which are also temporary but which do not manifest themselves until they have reached the next host. They include: (1) the deleterious effects of residence in the mosquito upon infectivity of sporozoites for the vertebrate host; (2) changes in the number of exoerythrocytic stages and of gametocytes which carry over to at least one subsequent passage; and (3) the effect of the vertebrate host upon the ability of the gametocyte to infect mosquitoes. The effects listed in this category are not essentially different from those in the first but merely represent instances in which a second host is needed to bring the effect on a particular stage into evidence.

The category for most lasting effects which may be exhibited through many passages in hosts includes: (1) the type of change described by Greenberg in which the characteristics of the strain are retained through many passages, even when alternating between vertebrate and mosquito hosts; and (2) the permanent loss in strains of the ability to produce gametocytes. These changes must be considered genetic either in the restricted meaning of the term to genic action or in the wider meaning of

any transmissible character regardless of the mechanism by which it is transmitted. Whether the host plays any role in influencing mutation rates in the parasites is questionable. Such a role has not been proved. Nor do we have any evidence for the type of rapid changes in antigenicity which results in certain organisms such as some spirochetes and trypanosomes in the presence of antibodies in the host. The apparently similar effects observed in drug fastness of malarial parasites fall outside the limits I have set for this paper but they may serve as useful tools in studying the results of hybridizations between different strains (see Trembley and Greenberg, 1954).

We may conclude that, in spite of the many interesting effects which are produced upon the parasite by the host, there is little evidence that any of these effects play any part in producing any lasting change in the behavior or structure of the parasite. It may even be that, because of their intracellular location, malarial parasites are less likely to be changed by the effects of humoral substances of large molecular size than extracellular parasites such as trypanosomes. The mechanism for alternating between vertebrate and invertebrate hosts which comes into play in the natural epidemiology of malarial infections, together with the presence of regular sexual reproduction, may possibly be effective in quickly disposing of mutations which are detrimental to the species.

REFERENCES

Barber, M. A. 1936. Degeneration of sporozoites of the malaria parasite in anopheline mosquitoes in nature and its relation to the transmission of malaria. *Am. J. Hyg.* **24**, 45-56.

Barzilai-Vivaldi, G. and Kauders, O. 1924. Die Impf-Malaria-experimentell durch Anophelen nicht übertragbar. *Wien. klin Wochenschr.* **37**, 1055-1057.

Boyd, M. F. 1945. On difficulties arising in the experimental propagation of falciparum malaria. *Am. J. Trop. Med.* **25**, 293-306.

Boyd, G. H., and Allen, L. H. 1934. Adult size in relation to reproduction of the avian malaria parasite, *Plasmodium cathemerium*. *Am. J. Hyg.* **20**, 73-83.

Brackett, S., Waletzky, E., and Baker, M. 1946. The relation between pantothenic acid and *Plasmodium gallinaceum* infections in the chicken and the antimalarial activity of analogues of pantothenic acid. *J. Parasitol.* **32**, 453-462.

Bray, R. S. 1958. Studies on malaria in chimpanzees. VI. *Laverania falciparum*. *Am. J. Trop. Med. & Hyg.* (In press)

Caldwell, F. E. 1944. *In vitro* effects of high temperatures on avian malarial parasites. *J. Infect. Dis.* **74**, 189-205.

Cantrell, W., and Jordan, H. B. 1946. Changes in the infectiousness of gametocytes during the course of *Plasmodium gallinaceum* infections. *J. Infect. Dis.* **78**, 153-159.

Chernin, E. 1952. The relapse phenomenon in the *Leucocytozoön simondi* infection of the domestic duck. *Am. J. Hyg.* **56**, 101-118.

Coatney, G. R. 1938. A strain of *Plasmodium relictum* from doves and pigeons infective to canaries and the common fowl. *Am. J. Hyg.* **27**, 380-389.

Cuboni, E. 1926. Gametenfreie *Plasmodium vivax*-Stämme. *Wien. klin. Wochenschr.* **39**, 1475-1476.

Dubin, I. N. 1954. Growth of exoerythrocytic forms of *Plasmodium gallinaceum* in epithelial cells in tissue culture. *Exp. Parasitol.* **3**, 425-444.

Eyles, D. E. 1951. Studies on *Plasmodium gallinaceum*. I. Characteristics of the infection in the mosquito, *Aedes aegypti*. *Am. J. Hyg.* **54**, 101-112.

Eyles, D. E. 1952a. II. Factors in the blood of the vertebrate host influencing mosquito infection. *Am. J. Hyg.* **55**, 276-290.

Eyles, D. E. 1952b. III. Factors associated with the malaria infection in the vertebrate host which influence the degree of infection in the mosquito. *Am. J. Hyg.* **55**, 386-391.

Gambrell, E. W. 1937. Variations in gametocyte production in avian malaria. *Am. J. Trop. Med.* **17**, 689-725.

Greenberg, J. 1955. Inheritance of certain characteristics by *Plasmodium gallinaceum*. *Trop. Med. & Hyg. News* **4**, 6-14.

Greenberg, J., Trembley, H. L., and Coatney, G. R. 1950. Strain differences in *Plasmodium gallinaceum* Brumpt. I. Differences in the behavior of the exoerythrocytic forms of a blood-passaged (BI) and sporozoite-passaged (SP) strain of *Plasmodium gallinaceum*. *J. Nat. Mal. Soc.* **9**, 320-326.

Greenberg, J., Trembley, H. L., and Coatney, G. R. 1951. Strain differences in *Plasmodium gallinaceum* Brumpt. IV. Experiences with blood passage of the phanerozoiteless M strain. *J. Nat. Mal. Soc.* **10**, 82-89.

Haas, V. H., Wilcox, A., Davis, F. P., and Ewing, F. M. 1946. *Plasmodium gallinaceum* infection characterized by predominance of exo-erythrocytic forms. *Public Health Repts.* **61**, 921-928.

Haas, V. H., Wilcox, A., and Ewing, F. M. 1945. Infection of chick embryos with non-pigmented forms of *Plasmodium gallinaceum*. *J. Nat. Mal. Soc.* **4**, 279-284.

Haas, V. H., Wilcox, A., Laird, R. L., Ewing, F. M., and Coleman, N. 1948. Symposium on exoerythrocytic forms of malarial parasites. VI. Response of exoerythrocytic forms to alterations in the life-cycle of *Plasmodium gallinaceum*. *J. Parasitol.* **34**, 306-320.

Huff, C. G. 1929. The effects of selection upon susceptibility to bird malaria in *Culex pipiens* Linn. *Ann. Trop. Med. & Parasitol.* **23**, 427-442.

Huff, C. G. 1930. Individual immunity and susceptibility of *Culex pipiens* to various species of bird malaria as studied by means of double infectious feedings. *Am. J. Hyg.* **12**, 424-441.

Huff, C. G. 1931. The inheritance of natural immunity to *Plasmodium cathemerium* in two species of *Culex*. *J. Prev. Med.* **5**, 249-259.

Huff, C. G. 1934. Comparative studies on susceptible and insusceptible *Culex pipiens* in relation to infections with *Plasmodium cathemerium* and *P. relictum*. *Am. J. Hyg.* **19**, 123-147.

Huff, C. G. 1940. Quantitative studies on size, variability, and growth rates of oöcysts of different strains of avian malaria. *Am. J. Hyg.* **32**, 71-80.

Huff, C. G. 1941a. Comparisons of a clon of *Plasmodium cathemerium* with its parent strain and with two strains derived from the clon by mosquito transmission. *J. Infect. Dis.* **68**, 184-187.

Huff, C. G. 1941b. Comparative importance of various factors upon the regulation of size of avian malarial oöcysts in mosquitoes. *Am. J. Hyg.* **34**, 18-21.

Huff, C. G. 1942. Schizogony and gametocyte development in *Leucocytozoön simondi*, and comparisons with *Plasmodium* and *Haemoproteus*. *J. Infect. Dis.* **71**, 18-32.

Huff, C. G. 1948. Natural immunity and susceptibility of doves and pigeons to exoerythrocytic and erythrocytic stages of *Plasmodium relictum*. *Proc. 4th Internat. Cong. Trop. Med. & Mal.*, 602-606.

Huff, C. G. 1951. Observations on the pre-erythrocytic stages of *Plasmodium relictum*, *P. cathemerium*, and *P. gallinaceum* in various birds. *J. Infect. Dis.* **88**, 17-66.

Huff, C. G. 1954. Changes in host-cell preferences in malarial parasites and their relation to splenic reticular cells. *J. Infect. Dis.* **94**, 173-177.

Huff, C. G. 1957. Organ and tissue distribution of the exoerythrocytic stages of various avian malarial parasites. *Exp. Parasitol.* **6**, 143-162.

Huff, C. G., and Coulston, F. 1944. The development of *Plasmodium gallinaceum* from sporozoite to erythrocytic trophozoite. *J. Infect. Dis.* **75**, 231-249.

Huff, C. G., Coulston, F., Laird, R. L., and Porter, R. J. 1947. Pre-erythrocytic development of *Plasmodium lophurae* in various hosts. *J. Infect. Dis.* **81**, 7-13.

Huff, C. G., and Gambrell, E. 1934. Strains of *Plasmodium cathemerium* with and without gametocytes. *Am. J. Hyg.* **19**, 404-415.

Huff, C. G. and Marchbank, D. F. 1955. Changes in the infectiousness of malarial gametocytes. I. Patterns of oöcyst production in seven host-parasite combinations. *Exp. Parasitol.* **4**, 256.270.

Huff, C. G., Marchbank, D. F., and Shiroishi, T. 1958. Changes in the infectiousness of malarial gametocytes. II. Analysis of the possible causative factors. *Exp. Parasitol.* (In Press)

Jeffery, G. M. 1951. Observations on a gametocyteless strain of *Plasmodium falciparum*. *J. Nat. Mal. Soc.* **10**, 337-344.

Knowles, R., and DasGupta, B. M. 1932. A study of monkey-malaria, and its experimental transmission to man. *Indian Med. Gaz.* **57**, 213-268.

Kopeloff, N. 1930. Inoculation malaria: Sexual and asexual strains. *Psych. Quart.* **4**, 290-294.

Lewert, R. M. 1950a. Alterations in the cycle of *Plasmodium gallinaceum* following passage through tissue culture. *Am. J. Hyg.* **51**, 155-170.

Lewert, R. M. 1950b. Alterations in the cycle of *Plasmodium gallinaceum* following passage through tissue cultures. II. Behavior of the strains during multiple passage through chicks. *Am. J. Hyg.* **51**, 178-193.

Lumsden, W. H. R., and Bertram, D. S. 1940. Observations on the biology of *Plasmodium gallinaceum* Brumpt 1935, in the domestic fowl, with special reference to the production of gametocytes and their development in *Aedes aegypti* (L). *Ann. Trop. Med. & Parasitol.* **34**, 135-160.

Manwell, R. D. 1943. Malaria infections by four species of *Plasmodium* in the duck and chicken, and resulting parasite modifications. *Am. J. Hyg.* **38**, 211-223.

McGhee, R. B. 1951. The adaptation of the avian malaria parasite *Plasmodium lophurae* to a continuous existence in infant mice. *J. Infect. Dis.* **88**, 86-97.

O'Roke, E. C. 1934. A malaria-like disease of ducks caused by *Leucocytozoön anatis* Wickware, *Univ. Mich. Sch. of Forestry & Conserv. Bull.* #4, 44 pp.

Plehn, A. 1925. Beobachtungen bei Paralytikermalaria. *Arch. f. Schiffs- u. Trop.-Hyg.* **29**, 56-65.

Porter, R. J. 1942. The tissue distribution of exoerythrocytic schizonts in sporozoite-induced infections with *Plasmodium cathemerium*. *J. Infect. Dis.* **71**, 1-17.

Redmond, W. B. 1943. Mosquito transmission of the pigeon strain of *Plasmodium relictum*. *Bull. Georgia Acad. Sci.* **1**, 9-10.

Redmond, W. B. 1944. Mosquito transfer of the pigeon strain of *Plasmodium relictum*. *J. Infect. Dis.* **74**, 184-188.

Rodhain, J. 1938a. Schizogonie sans pigment chez les pingouins. *Compt. Rend. Soc. Biol.* **127**, 368-372.

Rodhain, J. 1938b. Schizogonie sans pigment chez les pingouin; expérimentalement infecté de *Plasmodium praecox (relictum)*. *Compt. Rend. Soc. Biol.* **127**, 838-840.

Taliaferro, W. H. 1926. Variability and inheritance of size in *Trypanosoma lewisi*. *J. Exp. Zool.* **43**, 429-473.

Taliaferro, W. H., and Taliaferro, L. G. 1934a. The transmission of *Plasmodium faliciparum* to the howler monkey, *Alouatta sp.*, I. General nature of the infections and morphology of the parasites. *Am. J. Hyg.* **19**, 318-334.

Taliaferro, W. H., and Taliaferro, L. G. 1934b. Morphology, periodicity and course of infection of *Plasmodium brasilianum* in Panamanian monkeys. *Am. J. Hyg.* **20**, 1-49.

Taliaferro, W. H., and Taliaferro, L. G. 1944. The effect of immunity on asexual reproduction of *Plasmodium brasilianum*. *J. Infect. Dis.* **75**, 1-32.

Taliaferro, W. H., and Taliaferro, L. G. 1947. Asexual reproduction of *Plasmodium cynomolgi* in rhesus monkeys. *J. Infect. Dis.* **80,** 78-104.

Taliaferro, W. H., and Taliaferro, L. G. 1949. Asexual reproduction of *Plasmodium knowlesi* in rhesus monkeys. *J. Infect. Dis.* **85,** 107-125.

Thompson, P. E. 1944. Changes associated with acquired immunity during initial infections in saurian malaria. *J. Infect. Dis.* **75,** 138-149.

Thompson, P. E., and Huff, C. G. 1944. A saurian malarial parasite, *Plasmodium mexicanum* n. sp. with both elongatum- and gallinaceum-types of exoerythrocytic stages. *J. Infect. Dis.* **74,** 48-67.

Trembley, H. L., and Greenberg, J. 1954. Further studies on the hybridization of strains of *Plasmodium gallinaceum*. *J. Parasitol.* **40,** 475-479.

Trembley, H. L., Greenberg, J., and Coatney, G. R. 1951a. Strain differences in *Plasmodium gallinaceum* Brumpt. II. Experiences with the sporozoite and single oocyst passage of the BI strain. *J. Nat. Mal. Soc.* **10,** 68-75.

Trembley, H. L., Greenberg, J., and Coatney, G. R. 1951b. III. The spontaneous conversion of a phanerozoite-producing SP strain to a phanerozoiteless M strain through mosquito passage. *J. Nat. Mal Soc.* **10,** 76-81.

Wolfson, F. 1939. Morphological differences in *Plasmodium relictum* in canaries and ducks *(Anas boschas domestica). Am. J. Hyg.* **30,** Sect. C, 123-124.

Wolfson, F. 1940. Exo-erythrocytic schizogony associated with the wood-thrush strain of *Plasmodium cathemerium* in relation to the species of the host. *Am. Jour. Hyg.* **31,** Sect. *C,* 26-35.

HOST RESISTANCE TO THE KHARTOUM STRAIN OF *LEISHMANIA DONOVANI*

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Introduction

COMPARED with trypanosomiasis and malaria our knowledge of host resistance to leishmaniasis is quite in its infancy. This is also true of other aspects of research in this field and, except for the elucidation of the problem of transmission of *L. donovani* by sandflies, the last decade has seen no great advances in cultivation, serology, chemotherapy or experimental methods for the mammalian pathogenic strains of the parasite.

Evidence does exist, however, for host resistance to this group of organisms. Of the many kinds of animals into which *Leishmania,* from culture, insect gut or mammalian tissue, have been injected some become infected and some do not. Of those infected, there are even some claims for recovery (e.g. Laveran, 1920). Besides, in endemic areas all exposed animals do not become infected. In the human dermal infections, recovery, long known, obviously is a manifestation of acquired resistance. In the human visceral infections, the rarity of second cases, the appearance of post kala-azar dermal leishmanoid and the evidence for spontaneous cure are also considered examples of host resistance. Indeed, Kirk (1944), summarizing the work to that date, believes that known human visceral leishmaniasis may simulate any of the clinical aspects of both the dermal and the visceral infections. He suggests the following course as the basic infection: 1) *Primary stage,* with cutaneous sores at the site of inoculation having a tendency to spontaneous cure. 2) *Secondary stage,* a generalized infection like kala azar, and 3) *Tertiary stage,* the late cutaneous or mucocutaneous involvements.

Kirk points out that all these stages are not likely to be seen in any one individual infection and stresses that some strains of parasites show tendencies for predominant expression of one or the other of the stages listed. He claims the infection "may be terminated at any stage by the defense mechanism of the host gaining the upper hand, with the development of complete immunity and suppression of the parasites," or the parasite may gain the upper hand with the death of the host.

Finally, there is the serological evidence. The reviews of Taliaferro

(1929) and Culbertson (1941) contain numerous references to the presence of and *in vitro* action of antibodies associated with natural infection or produced as a result of the artificial immunization of animals. Some of these, like the formol gel test, are based on non-specific alterations of the plasma proteins; others, like the Witebsky, Klingenstein and Kuhn test, utilize a bacterium as the antigenic source. It appears generally agreed that not one of these antibodies has been proven to play any significant role in host resistance, innate or acquired. Even in *L. tropica* infections, where resistance to reinfection is best established, the nature of antibody action, if any, has not been disclosed. The nearest to the demonstration of true host sensitization in infection is implied by the Montenegro test for American mucocutaneous leishmaniasis.

Although some good work has been done with animal infections, no attempt will be made here to review this large literature. Many of the observations are casual ones; few are at all quantitative. Besides, Kirk's statements bear repetition. He injected dermal and visceral parasites by various routes into white mice and monkeys. "The results were so variable and included so many failures to produce any type of infection that no useful information regarding differentiation of strains has been obtained by this method. Perusal of the literature shows that other workers have had similar experiences. Differentiation of strains by animal inoculation is difficult unless large numbers of suitable animals are available" (see also Harrison and Fulton, 1946). On this basis it is difficult to evaluate even the few claims for recovery from leishmaniasis in susceptible hosts.

The above considerations are not meant to imply that there are no differences among the species or strains of *Leishmania* infecting mammals. However, taken together with the evidence that in most endemic areas leishmaniasis is a zoonosis, the implication is strong that they are a closely related group of parasitic organisms. As Cameron (1956) puts it "The Leishmanias, it would seem, are species which are still in the making."

For these reasons the present discussion will be limited to the study of the recent work on the Khartoum strain of *L. donovani* in experimental animals in our laboratory. While the limitations of such a procedure are obvious, the methodology to be described and the results so far obtained suggest that a beginning has been made toward furnishing an experimental basis for the elucidation of the problems of host resistance to the genus *Leishmania*. Even though only a single strain of one species has been studied, Kirk's requirement has been met in part since the statements made are based on information gleaned from the study of visceral leishmaniasis in more than 5,000 laboratory animals over the past few years.

Materials and Methods

The following animals have been used as hosts: laboratory white rat (Long-Evans and Wistar strains), white mouse (CF_1 strain), guinea pig, rabbit, chinchilla (*Chinchilla lanigera*), cotton rat (*Sigmodon h. hispidus*), golden hamster (*Mesocricetus auratus*) and Mongolian gerbil (*Meriones unguiculatus*).

In the work to be discussed here all inoculations of parasites were made intravascularly and in most cases the unsuitability of superficial veins led to the intracardial route as the routine one. The inoculum was prepared by grinding infected spleen (or occasionally liver) in sterile saline in a Ten Broeck tissue grinder. Dilutions were made in saline or occasionally in serum-saline.

Parasites were counted by two methods:

1) from Giemsa-stained organ impression smears, as parasites per organ-cell nucleus. Counts to 1,000 organ cell nuclei were made except where the ratio was greater than 3 parasites per organ cell nucleus. At such high levels of parasitization, counts were made only to 1,000 parasites.

2) from smears made from suspensions prepared by grinding in saline approximately 100 mg. of organ in the tissue grinder and spreading 0.005 ml. of this suspension on a slide over an area of approximately 1 cm. square. After staining with Giemsa, the parasites were counted in a sample of the material spread on the slide and an estimate obtained of the number of parasites per milligram of infected organ. On multiplying either the ratio obtained with method 1 or the density per mg. obtained with method 2 by the weight in milligrams of the infected organ, a figure is obtained which is indicative of the number of parasites in the whole organ. There is a high degree of correlation between the counts obtained by the two methods and the relationship between them suggests that each unit obtained from the organ impression smear (method 1) represents approximately 200,000 parasites.

We accept and confirm the statistical evaluation of such counting procedures as described by Goodwin (1944) and Fulton, Joyner and Chandler (1950). We have also considered the influence of such factors as organ hyperplasia on the counts obtained. The high correlation between the counts obtained by the two methods, one independent of organ cell complexity, has given assurance that no further corrections are necessary for the data as used in this study.

Since method 1 is the easier to perform, it is the one which has been usually followed in this work. Previous work (Stauber, 1955) has shown that in infections produced by intravascular inoculation better than 95% of the parasites injected are found in the liver within an hour. This pro-

portion remains true for the early part of the infection but because the rate of increase in the spleen remains higher than in the liver, the total number of parasites in the spleen finally approaches that in the liver if the animal lives long enough. No other organ examined in these infections regularly contains appreciable numbers of parasites, except the bone marrow. Furthermore, since we do not yet have a method of satisfactorily determining the total numbers of parasites in the bone marrow, the parasites in liver and spleen are considered adequate expressions of the total parasite burden of the host.

The threshold of patency by method 1 is one parasite per thousand organ-cell nuclei. Calculated as described, this amounts to approximately 200 parasites per milligram of organ being sampled. Consequently, not finding a parasite under the procedure outlined means merely that less than 200 parasites per mg. were present. Applied to whole organs of some of the animal species studied these thresholds are approximately 25,000 parasites for mouse spleen, 19,000 for hamster spleen, 200,000 for rabbit spleen, 450,000 for gerbil liver, 700,000 for hamster liver and 2,000,000 for chinchilla liver. We have not resorted to cultivation or subinoculation procedures to establish densities lower than this, though the culture method of Germuth, Eagle and Oyama (1950) appears nearly able to detect a single organism and we believe we can do this by intracardial subinoculation in the hamster. Because of the failure to obtain values below the thresholds indicated, certain assumptions are made for a few of the points in Tables II and III and Figs. 1 and 3, but these are obvious.

Results

The primary infection in the hamster. The principal aspects of this infection as we see it in our laboratory have already been published and the variability of the responses has been noted (Stauber, 1955; Stauber, Franchino and Grun, 1958). After intracardial inoculation of a few million parasites, the number of organisms increases initially, then less and less sharply, but continue to rise until the death of the host 35-45 days later. With such large inocula, as mentioned previously, the number of parasites in the spleen at death does not equal that in the liver. These differences in rates of accumulation of parasites in spleen and liver are not yet understood but are made evident in the data in Table I. In this experiment tenfold dilutions of inoculum to contain from 5,000,000 to 0.05 parasites per 0.1 ml. when injected, resulted in infections whose duration varied inversely with the number of parasites in the inoculum. It appears that as little as one parasite may produce a fatal infection in the hamster though it may take from 169 to more than 251 days to reach the fatal consequence. Such being the case, it appears that the hamster

shows neither innate nor acquired resistance to the parasite. It should be noted, however, that if the injection of parasites is made intraperitoneally evidence of what might be called one aspect of innate resistance can be obtained. It requires ten times as many parasites intraperitoneally as intracardially to produce a median time to death comparable to group I in Table I. It requires 2500 times as many parasites intraperitoneally to duplicate a median time to death comparable to group IV in the same

TABLE I

TERMINAL FINDINGS IN HAMSTERS AFTER INTRACARDIAL INJECTION OF DIFFERENT NUMBERS OF *L. donovani*

Group No.	Inoculum Size*	Median time to Death (Days)	Total parasites Spleen (in Millions)	Total parasites Liver (in Millions)	Ratio Parasites-Liver Parasites-Spleen
I	1×10^{0}	37	311	2,182	7.0
II	1×10^{-1}	47	246	1,101	4.5
III	1×10^{-2}	64	475	1,438	3.0
IV	1×10^{-3}	82	561	439	0.8
V	1×10^{-4}	116	301	643	2.1
VI	1×10^{-5}	123	671	861	1.3
VII	1×10^{-6}	161	102	176	1.7
VIII	1×10^{-7}	169†	288	82	0.3‡
IX	1×10^{-8}	251†	—	—	—§

* Expressed as dilutions of initial suspension. The undiluted ($=10^{0}$) contained 5,000,000 leishmania per inoculum of 0.1 ml.

† Two of eight animals in group VIII and 7 of 8 animals in group IX showed no parasites at 251 days after inoculation. This approximates the chances that, at such dilution, inoculum did not contain a parasite.

‡ Based on only one animal dead of the infection.

§ One animal infected—not dead at 251 days when experiment was ended.

table. These represent enormous losses of parasites upon intraperitoneal injection. Similar results are obtained by subcutaneous injection of parasites. Although we have made no extensive study of this, the results, viewed in the light of Table I, would seem to indicate that only about 500 of the 40 million parasites injected survived. This information may explain in part the greater variability of results obtained by various investigators using other than intravascular inoculation (Kirk, 1944; Harrison and Fulton, 1946).

The primary infection in the gerbil. The course of infection in the gerbil also depends on the size of the inoculum injected. This is shown in Fig. 1 where each point represents the median parasite count in a group

of seven animals. When large numbers of organisms (40,000,000 and 2,000,000 parasites) are injected, a 2½- to 3½-fold rise to a peak occurs in the liver followed by a decline in numbers to levels below those at two hours after inoculation. The decline begins earliest for the group receiving the largest inoculum. When smaller numbers of parasites are injected (200,000), although the data are less significant since in the early period

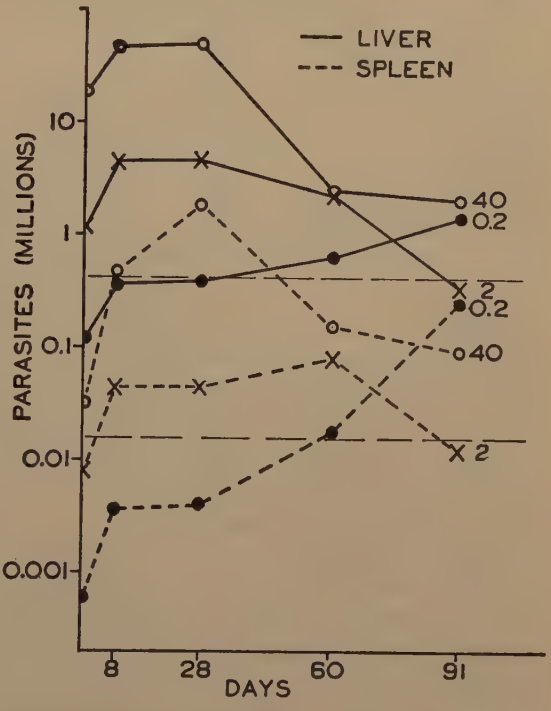

FIG. 1. Course of infection with *L. donovani* in the gerbil determined by parasite counts in liver and spleen after intracardial injection of 40 million, 2 million or 0.2 million parasites. (indicated by numbers at right). Each point on a curve is the median value for a group of seven animals except for the 91st day (only 3 animals). The two horizontal lines of dashes represent the thresholds of patency for liver (upper) and spleen (lower) respectively.

of the infection a number of the animals in each group never reach a condition of patency, the rise to the peak takes a much longer time. Similar number curves are seen in the spleens of the same animals. The spleen counts also show, as in the hamster, less than 5% of the number of parasites found in the liver of the gerbil at 2 hours and 8 days after inoculation.

Figure 2 shows the mean spleen weight/body weight ratios for the animals in this experiment and, together with Fig. 1, shows the correlation between the numbers of parasites injected, the course of the infection, and the amount of splenic hypertrophy induced.

These data indicate that although the gerbil is susceptible to infection and is a host suitable for multiplication of the parasite within it, true

acquired resistance occurs which leads to the virtual, but not necessarily complete, elimination of the parasites from the gerbil.

Comparative host resistance. As indicated above, the methods employed are adequate to disclose differences in the responses of different hosts to infection with *L. donovani.* Furthermore, the reproducibility of

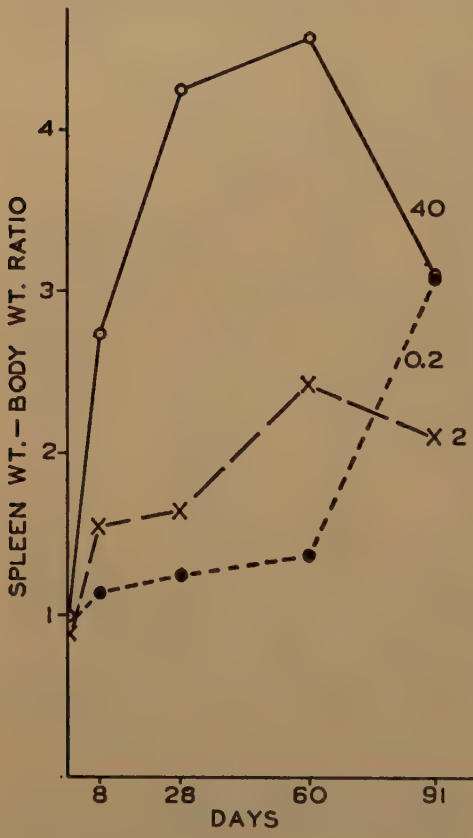

Fig. 2. Changes in group median spleen wt./body wt. ratio for the same gerbils seen in Fig. 1 at various times after inoculation with 40, 2 or 0.2 million parasites (indicated by numbers at right).

the results of an experiment is of a high order. For example, in an experiment similar to that described in Table I but performed three years previous, almost identical results were obtained.

To test for differences in susceptibility and resistance, groups of animals of the eight species mentioned previously were infected under roughly standard conditions of route and size of inoculum (part of this work has already been reported by Grun and Stauber, 1957). The inoculum size was approximately of the order of 20,000-500,000 parasites per gram of body weight. Groups of animals so infected were sacrificed at

intervals after inoculation; typical times were 1-2 hours, 1 day, 4, 8, 16, 28, 60, 120 and more days, depending on the characteristics of the infection. Usually 4-8 animals constituted a group. Not all these samplings were obtained in a single experiment but subsequent experiments filled in the gaps of information sought. On necropsy, body, spleen and liver weights and spleen and liver impression smears were obtained. Homogenate smears (method 2) were made only early in each series to furnish the necessary comparison material. This was true also for blood and bone marrow (femur) smears.

Because the final ranges of parasite numbers in the combined data extend over so many logs of numbers (1×10^0 to 1×10^{10}) and the important points on the time scale extend from a few days to more than 6 months, the data in Tables II and III have been expressed in the form of multiples of parasite increase for a few specific periods of time. These periods of time are somewhat arbitrarily chosen since, except perhaps for the first eight-day period for many of the species, no other points of time are correspondingly significant for more than one or two species. Besides, the points of time used for the liver do not always characterize entirely similar parts of the course of infection occurring in the spleen. Finally, all the brackets of time are not included for each species because of the death or recovery of the host. A log-log plot of the data (Fig. 3), although it, too, has limitations, is also helpful in comparing the results.

In spite of these limitations a number of interesting points concerning host resistance are evident from Tables II and III and Fig. 3. A great range from high susceptibility to almost complete resistance is shown with further variations within the categories. If innate resistance is defined as the unsuitability of the host as a culture medium for the parasite, its capacity to destroy the introduced parasite before the production and mobilization of antibodies and special cellular response, or both combined, then the changes in parasite numbers over the first 8 days may be an adequate relative expression of this. On this basis the animals shown in Tables II and III and Fig. 3 fall into 3 groups. (Since the time brackets in the tables are different, comparisons should only be made in the vertical columns.) The hamster and cotton rat are excellent hosts, there being usually about 50 times as many parasites at 8 days as there were at 1 hour after inoculation. The chinchilla, mouse, gerbil and guinea pig are moderately good hosts and the rabbit and rat are quite unsuitable. There is a possible error of interpretation here, applicable to any of the species, since some of the parasites observed at 1-2 hours may already be dead. This would falsely lower the fold increase for the 8-day period, though it would be an expression of a kind of host resistance. In the experiments

TABLE II

THE COURSE OF INFECTION WITH *Leishmania donovani* IN THE LIVERS OF A SERIES OF EXPERIMENTAL ANIMALS AFTER INTRAVASCULAR INOCULATION OF PARASITES

Animal Host	Changes in Parasite Densities with Time			
	1 Hour to 8 Days	8 Days to 62 Days	62 Days to End of Exp.	1 Hour to Peak Day
Cotton Rat	44	61	2.9 (196)	7,784 (196)
Hamster	50	100 (62)	>1.0	>5,000 (>62)
Chinchilla	3	50	1.9 (120)	285 (120)
Mouse	5	0.5	0.9 (90)	5 (8)
Gerbil	3	0.5	0.1 (143)	6 (28)
Guinea Pig	7	<0.04	—	13 (16)
Rabbit	0.8	0.07	—	1.2 (4)
Rat	0.4	<0.02	—	0

Numbers in parentheses are days to end of experiment or to peak of infection. All other numbers are expressed as multiples of the value at the beginning of the time interval. For the cotton rat, as expressions of rates of reproduction, the multiples in the 3 time intervals divided by the number of days in the interval, give values of 5.5, 1.2 and 0.02, which therefore represent the mean change in parasite density per day in the respective periods. The data enclosed in the block represent moderate susceptibility, those above it relatively high susceptibility, and those below it relatively low susceptibility. It will be seen that these degrees of susceptibility are not the same in all three time intervals.

TABLE III

THE COURSE OF INFECTION WITH *Leishmania donovani* IN THE SPLEENS OF A SERIES OF EXPERIMENTAL ANIMALS AFTER INTRAVASCULAR INOCULATION OF PARASITES

Animal Host	Changes in Parasite Densities with Time			
	1 Hour to 8 Days	8 Days to 62 Days	62 Days to End of Exp.	1 Hour to Peak Day
Cotton Rat	17	1,863	10.6 (196)	335,000 (196)
Hamster	100	1,000 (62)	>1.0	>10,000 (>62)
Chinchilla	3	300	20 (120)	17,500 (120)
Mouse	3	15	1.0 (90)	46 (36)
Gerbil	15	7	0.1 (143)	172 (44)
Guinea Pig	4	0.2 (38)	—	4 (8)
Rat	1.8	<0.5 (23)	—	4 (6)
Rabbit	1.5	<1.1 (22)	—	5 (4)

Numbers in parentheses are days to end of experiment or to peak of infection. All other numbers are expressed as multiples of the value at the beginning of the time interval. For meaning of blocks see legend for Table II.

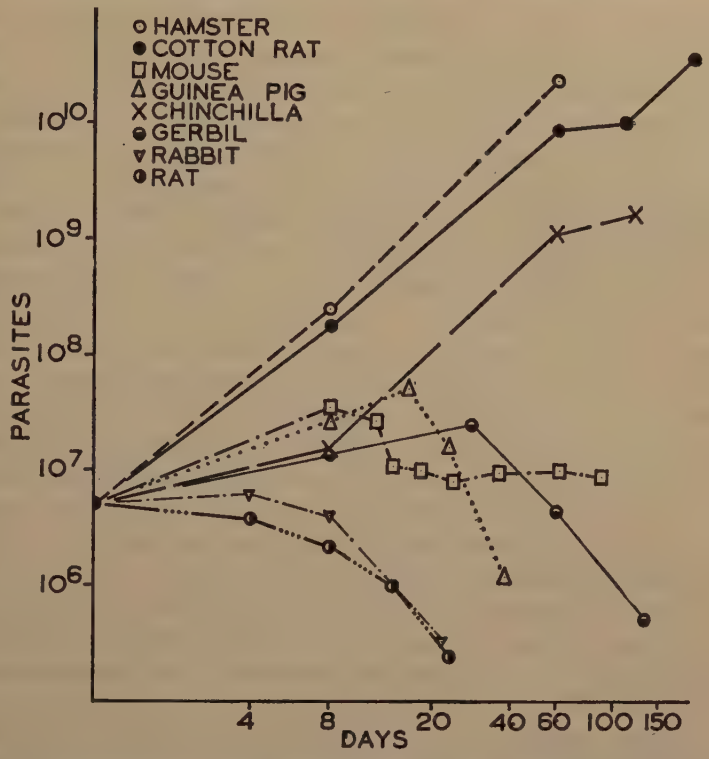

Fig. 3. Representative courses of infection in eight species of small mammals as determined from parasite counts in the liver on various days after inoculation with *L. donovani*. All data adjusted to approximately the same initial density of parasites in the liver at 1 to 2 hours after inoculation. The threshold of patency for these conditions lies close to the axis for 1×10^5 parasites.

cited, however, this factor does not seem to be important (1) because the per cent recovery of parasites at 1-2 hours seems to be similar (60-75%) for all species and (2) no significant decrease in parasite numbers occurs within the first 24 hours to suggest relatively quick digestion of parasites dead when phagocytized. In fact, in all susceptible species, there is evidence for an increase in parasite numbers within the 24-hour period.

In the second period of time (8 to 62 days), it is clear that the chinchilla now approaches the cotton rat and the hamster in susceptibility and is very unlike the mouse, gerbil and guinea pig which it first resembled. It should be noted that the higher numbers in this period of time for these 3 animal species, as seen in the tables, and the steeper slope in Fig. 3 for the chinchilla, do not mean increased rates of multiplication over those of the 1-hour to 8-day period. Actually these rates are less in the 8 to 62 day period, except possibly for the chinchilla, since the total

increases shown are for only 8 days in the first period, and for 54 days in the second period. The guinea pig in the 8 to 62 day period shows a number peak and a sharp decline. Its over-all response for this period is very much like that of the resistant rat and rabbit though in the case of the guinea pig this must be acquired resistance since appreciable multiplication occurs up to day 16. The mouse remains similar to the gerbil in this period, reaching a low peak and declining slightly.

In the final period (beyond 62 days), the number of parasites continues to increase in the cotton rat and chinchilla, though the rate of increase is greatly lessened. A hamster surviving into this period also continues to show an increasing parasite burden but when inoculated with the number of parasites used here (approximately 120,000 parasites per gram body weight) it rarely survives the midpoint of this time bracket. In order to obtain infections which will allow survival as long as this (like group III of Table I) much smaller numbers of parasites must be injected. Since the hamster dying of leishmaniasis has approximately the same number of parasites in its organs regardless of size of inoculum (Table I), the number for the fold increase in this period will thus depend somewhat upon the number of parasites injected. The figures in Tables II and III for the hamster are, then, neither exact determinations nor exactly comparable to the other data but are the best compromises that could be obtained.

In this final period the mouse shows more resemblance to the cotton rat and chinchilla than to the gerbil, which it resembled earlier. It continues to show significant numbers of parasites and to remain patently infected as long as examined (up to 90 days). However, since the level of parasitization does not increase, an acquired resistance is manifest, although we are yet unable to say whether only destructive forces are producing this effect or whether inhibition of reproduction of the parasite occurs as well.

The gerbil's response in this final period is that of an animal which has acquired a significant level of resistance (see also Fig. 1). The parasite burden is reduced almost to zero and thus it comes finally to resemble the guinea pig. Concerning the latter, as well as the rabbit and the rat, no further statements are possible since the parasite level is reduced below the threshhold of patency long before the beginning of this period.

The final column is the total fold increase to peak day. For hamster, chinchilla and cotton rat no cessation of parasite reproduction occurs, so there appears to be little evidence of any effective host resistance at all. The rate of accumulation of the parasites does, however, decrease at each successive interval after 8 days; whether this is a partial manifes-

tation of acquired immunity or an athrepsis cannot be decided as yet. It may be that the host fails to supply the requirements for the parasites at a rate equal to the parasite's potential for reproduction. The subtle difference between the findings in spleen and liver suggest that the problem is more complex than implied by such an hypothesis.

Since the hamster and chinchilla are susceptible to the "toxic" or debilitating influences of *L. donovani*, they eventually die. The cotton rat, tolerant or indifferent to these influences, remains alive, without symptoms, presumably as long as it lives (Fulton and Niven, 1951), a response very much like that described years ago for the Chinese hamster (Meleney, 1925).

A few added comments are necessary regarding some of the differences between the results recorded for spleen and liver in Tables II and III. In the first time period the high multiple of 15 for the gerbil spleen seems appreciably different from the figures for others in the moderately resistant group, but no explanation is available.

In the period from 8 to 62 days in the spleen of the susceptible cotton rat, hamster and chinchilla, the rates of increase of parasites were about 10 times as great as in the liver during the same period. They were still not as great, however, as the rates of increase during the period from 1 hour to 8 days in the spleen or in the liver.

The very large numbers shown in the final column of Table III (1 hour to peak day) for cotton rat, hamster and chinchilla bear out the statements made previously concerning the initial and final totals of parasites observed in the spleens of these animals. This would indicate that in some unknown manner the spleen is a more hospitable organ for the multiplication and accumulation of parasites even though (or because?) it initially receives many times fewer organisms.

Superinfection. In the chinchilla repeated superinfection (as many as 6 times) during the course of an infection, with numbers of parasites small in comparison with those already accumulating in its organs, has been tried. No alteration of the course of the initial infection could be detected.

In the hamster the experiments were run differently. A small initial infecting dose of parasites was given so that, at a later date, superinfection could be obtained with such a significantly higher number of parasites that the two infections could be distinguished. For the initial infection the number of parasites given was below the threshold of patency until 8 or 16 days later, at the time of superinfection. This gave the host an appreciable time of contact with the parasite, presumably time in which to respond with any available mechanisms for resistance.

A typical experiment is shown in Fig. 4. The initial infection was 300,000 parasites and the superinfection 47,000,000 parasites. Some control groups of animals were given only the superinfecting dose, other controls were not superinfected at all, and a final control group, pre-

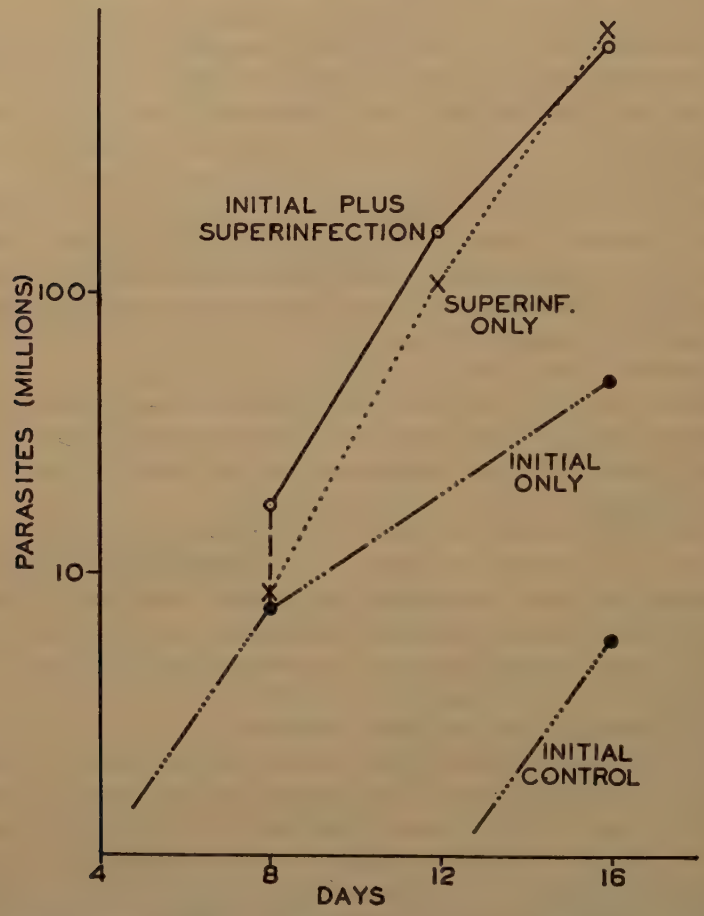

Fig. 4. Superinfection of hamsters with *L. donovani*. Superinfection performed on 8th day after beginning of the experiment.

viously uninfected, received at the time of the superinfection the same number of parasites as the animals initially infected (namely 300,000). It is obvious from Fig. 4 that the rate of parasite increase in the superinfected animals was about as great as in those animals receiving such a large number of parasites for the first time. On the other hand, the rate of increase of parasites in the non-superinfected control group showed

the decrease in rate expected for the period beyond 8 days, as already discussed in connection with Table II.

If the normal decrease in rate of accumulation of parasites as the infection progresses is a measure of a partially effective acquired resistance, these results might be explained by hypothesizing that the superinfection desensitizes the hamster back to its original susceptibility. At any rate, as in the chinchilla, there is no evidence that the ultimate outcome of the infection (namely death) is altered by the superinfection or by this supposed partial resistance.

In a series of experiments where successful treatment (with stibogluconate) of a heavy infection was followed by reinfection, ultimate death from leishmaniasis occurred in about the time expected on the basis of the size of the reinfecting dose. Also no kind of non-specific (ground celluflour, tuberculin, foreign red cells, foreign serum, india ink, ground uninfected liver and spleen suspensions, Newcastle virus vaccine) or specific (suspensions of killed leishmania in ground infected spleen or liver, killed leptomonads) stimulation of the hamster has afforded any significant measure of protection against the subsequent introduction of live parasites. The only conclusion from these experiments is that the hamster acquires little resistance to *L. donovani.*

It seems logical that an animal with such high susceptibility as the hamster or such high resistance as the rat would be unsuited to demonstrate appreciable heightened resistance to re-exposure. On the other hand, the gerbil would appear well suited for the demonstration. It is susceptible enough to allow parasite multiplication for a number of days (Figs. 1 and 3; Tables II and III) yet it ultimately eliminates nearly all the parasites which have so accumulated. Up to now only a single trial has been completed but the results of that superinfection experiment are found in Figs. 5 and 6. As controls, the pooled data obtained in other experiments with primary infections in the gerbil are presented. There are obvious objections to this procedure but it is the best we have to offer at this time. The inocula are all prorated to the same infecting dose to aid visual comparison.

In the experiment as conducted, 94 gerbils were infected with 10 million parasites and the course of this infection was followed by sampling at 2 hours, 8, 29, 60, and 120 days after inoculation. On the 60th day, when the sample counted already showed marked reduction of the parasites from the peak which occurred on or about the 28th day, 70 animals were superinfected with 30 million parasites. Sample counts were made from groups of seven each of the superinfected animals at 2 hours, 4, 8, 16, 24, 31 and 60 days after this second injection. It appears that a previous infection does increase the gerbil's capacity to destroy more rapidly para-

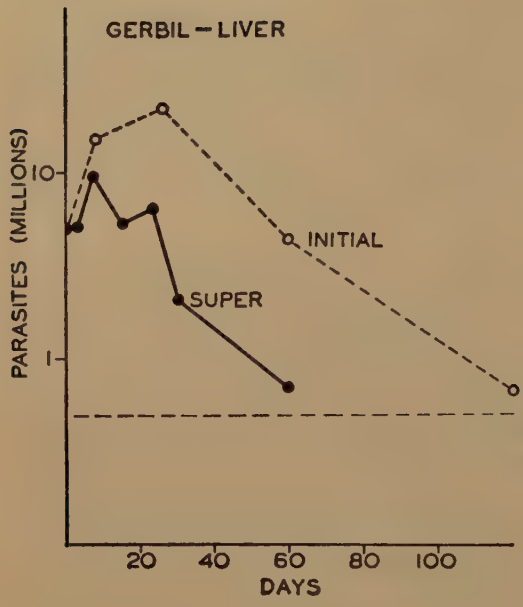

FIG. 5. Initial and superinfection of the gerbil with *L. donovani* as determined from parasite counts of liver impression smears. Curve for the initial infection from pooled data. Curve for the superinfection from a single experiment only (8 animals per group). Initial infection: 10 million parasites; superinfection: 30 million parasites. Curves adjusted to same density of parasites at 1 hour after inoculation. Horizontal line of dashes is the threshold of patency for the gerbil liver.

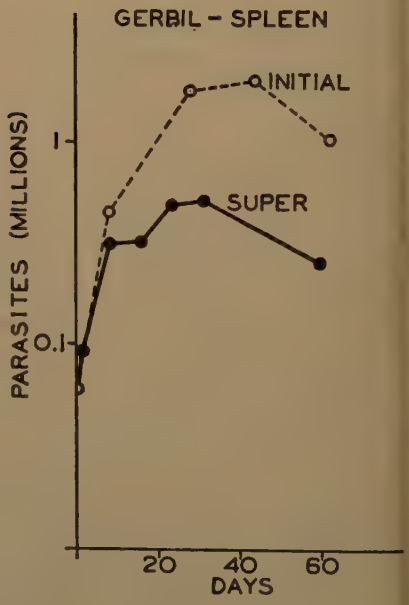

FIG. 6. Same as Fig. 5, initial and superinfection of the gerbil as determined by parasite counts of spleen impression smears. Base line of figure is the threshold of patency for the gerbil spleen.

sites which are introduced at a later time. Since parasite multiplication occurs at almost the rate seen in the primary infection, at least for a few days, it is obvious also that the degree of resistance afforded by a single previous infection is not very high. Repeated superinfections have not yet been tried. It is not yet known whether the apparent acquired resistance is an infection-type immunity based on the continued presence of the parasite, or whether true residual immunity occurs.

Conclusions and Summary

A whole range of resistance to *L. donovani* has been found within the series of animal species studied. It extends from the complete susceptibility of the hamster which eventually succumbs to the intracardial introduction of a single parasite, to the innately resistant rat and rabbit which soon dispose of even as many as several million parasites.

The chinchilla and cotton rat are highly susceptible also. The chinchilla dies of the infection, but the cotton rat is able to bear an enormous burden of parasites with no apparent ill effects.

In terms of acquired resistance, the mouse, gerbil and guinea pig are the most interesting species because each is susceptible enough to permit some increase in parasites for a number of days after inoculation, but each later checks this increase. The mouse apparently does not successfully eliminate the leishmania from its body, the parasite density remaining above the threshold of patency for many days.

The gerbil and the guinea pig do effect virtual removal of the parasites; the guinea pig accomplishes this result in about one-fourth the time that the gerbil requires.

Superinfection of the hamster and chinchilla show no detectable increase in resistance resulting from the prior infection. The gerbil, however, shows a measurably heightened resistance to a second infection.

The mechanisms responsible for the tolerance of the cotton rat to the damaging effects of large numbers of parasites, for the innate resistance of the rabbit and rat, or for the acquired resistance of the gerbil and guinea pig are still unknown.

Acknowledgments

This investigation was supported by a research grant (E-92) from the National Institute of Allergy and Infections Diseases, Public Health Service.

The author is greatly indebted to a number of his students for their assistance in this work. Special credit is due to John Grun, since a portion of this material will appear in thesis form under his name (see also

reference: Grun and Stauber, 1957). Others who merit mention are Paul Actor, Elizabeth M. Franchino and Richard N. Rossan. Thanks are due the West Foundation, Brant Lake, N.Y., for generosity in supplying most of the gerbils used in the work reported here.

REFERENCES

Cameron, T. W. M. 1956. *Parasites and parasitism.* Methuen & Co., Ltd., London.

Culbertson, J. T. 1941. *Immunity against animal parasites.* Columbia Univ. Press, New York.

Fulton, J. D., Joyner, L. P., and Chandler, R. L. 1950. Studies on protozoa. II. The golden hamster *(Cricetus auratus)* and cotton rat *(Sigmodon hispidus)* as experimental hosts for *Leishmania donovani. Trans. Roy. Soc. Trop. Med. & Hyg.* **44**, 105-112.

Fulton, J. D., and Niven, J. S. F. 1951. Studies on protozoa. III. Visceral leishmaniasis in the cotton rat *(Sigmodon hispidus). Trans. Roy. Soc. Trop. Med. & Hyg.* **44**, 717-728.

Germuth, F. G., Jr., Eagle, H., and Oyama, V. 1950. An evaluation of the criteria of cure in experimental leishmaniasis of the golden hamster. *Amer. J. Trop. Med.* **30**, 377-385.

Goodwin, L. G. 1944. The chemotherapy of experimental leishmaniasis. I. The spleen as an index of infection in the Syrian hamster. *Trans. Roy. Soc. Trop. Med. & Hyg.* **38**, 151-160.

Grun, J., and Stauber, L. A. 1957. A comparative study of experimental leishmaniasis in the mouse, Mongolian gerbil, hamster, white rat, cotton rat and chinchilla. *J. Parasitol.* **43** (Suppl.), 16.

Harrison, C. V., and Fulton, J. D. 1946. The effect of treatment on the spleen of the golden hamster *(Cricetus auratus)* infected with *Leishmania donovani. Brit. J. Exp. Pathol.* **27**, 1-8.

Kirk, R. 1944. Studies in leishmaniasis of the Anglo-Egyptian Sudan. VI. The evolution of the Leishmania infections in man. *Trans. Roy. Soc. Trop. Med. & Hyg.* **38**, 61-70.

Laveran, A. 1920. La leishmaniase canine chez la souris. *Bull. Soc. Pathol. Exot.* **13**, 680-687.

Meleney, H. E. 1925. The histopathology of kala azar in the hamster, monkey and man. *Am. J. Pathol.* **1**, 147-168.

Stauber, L. A. 1955. Leishmaniasis in the hamster. *In* Cole, W. H. *Some Physiological Aspects and Consequences of Parasitism.* Rutgers Univ. Press, New Brunswick, N.J.

Stauber, L. A., Franchino, E. M., and Grun, J. 1958. An eight day method for screening compounds against *Leishmania donovani* in the golden hamster. *J. Protozool.* **5**, (in press).

Taliaferro, W. H. 1929. *The immunology of parasitic infections.* The Century Co., New York.

INVASIVENESS OF HELMINTH LARVAE*

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THE invasions of tissues and cells by animal parasites are often spectacular processes when compared to the invasions of the host by bacteria. In some instances these invasions demand prolonged tissue migrations before the invading organism reaches the site within the host where it may survive and develop to maturity. With regard to bacterial invasion we have well documented evidence concerning many of the mechanisms involved and a basis for understanding the variations in the ability to invade found in different groups or strains of organisms. These have been related to toxin and enzyme production or to the possession by microorganisms of substances tending to negate host defenses. However, as a result of our lack of information none of the basic texts dealing with parasitism by animal agents includes specific discussion of this obviously fundamental process in successful parasitism of a host. Invasiveness of parasites in the broadest sense has been discussed by Taliaferro (1). He has pointed out that the invasiveness of parasitic organisms is a summation of a heterogeneous collection of factors which include specialized structures and specific biochemical entities produced by the parasite. Relative invasiveness of a parasite must also take into consideration the diverse host factors tending to resist invasion. The term invasiveness also implies that the invader is able to survive and reproduce. The following discussion will be limited to evidence and speculations concerning the mechanisms involved in the direct invasion of tissues by helminth larvae and to those host responses which may be related to these mechanisms.

The invasion of intact epidermal or mucosal membranes of the host followed by extensive migration through the deeper tissues is an essential portion of the life cycle of many helminth parasites. There exists a relative abundance of detail on the course taken by invading larvae during migration and development in both definitive and intermediate hosts. There is also a considerable literature describing histologic and cellular

* The work cited in this article was done in part under research grant G 4056 from the National Microbiological Institute of the National Institutes of Health, U. S. Public Health Service, in part under the sponsorship of the Commission on Parasitic Diseases, Armed Forces Epidemiological Board with support from the Office of the Surgeon General, Department of the Army, and by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

changes in the host as a result of or in response to these tissue invasions. However, our knowledge of the precise mechanisms which enable helminth larvae to penetrate tissues and migrate through them is relatively meager. Correlatively our understanding is slight of those hosts responses which are directed specifically against these mechanisms.

In some groups of helminth larvae, as in the stylet cercariae, it is probable that the major mechanism of invasion is mechanical, aided by secretory processes. In others, as in the filariae, the initial site of entry into the host may be by way of a break in the epithelium made by an agent other than the parasite. There are, however, a large number of tissue-penetrating helminths which are ill equipped for penetration of intact tissue barriers by physical means alone, although muscular locomotor activity is an integral part of the process. Some of these such as the *schistosomes, Strongyloides, Ancylostoma,* and their relatives, are readily capable of passing through the epidermis and into the dermis in a matter of a few minutes. Glandular structures that might secrete histolytic or other substances enabling the parasite to penetrate tissues are prominent features in many of these forms. In numerous instances where alteration of host tissues has been observed a causal relationship has been ascribed to the secretions of glands of larvae, adults, or embryonated eggs in the tissue (2-11). Until relatively recently, all of our information on parasite-induced changes in the host occurring during penetration or tissue migration has been based on indirect evidence of this nature. The first exception to this was the observation by Davis in 1936 (12) that a homogenate of a dermatitis-producing cercaria (*Diplostoma*) possessed a proteolytic activity that would cause "pulping" of bits of frog muscle. A similar activity was reported in 1937 by Hunter (13) for the cercariae of *Cryptocotyle.*

More recently a number of investigations have been made of parasite secretions and host-parasite interaction which give us the beginning of a more detailed concept of the nature of the enzymatic invasion mechanisms of helminths and the host response to these processes. These investigations have been more or less directly stimulated by advances in our knowledge of bacterial invasiveness as well as by the development of methods and advances made in histochemistry, microchemistry and immunology.

The first of these enzymatic mechanisms of invasion to be discussed is one in which the activity of certain larvae is to a great extent directed against the acellular constituents and intercellular cement substances of the tissues. During their brief and often rapid passage through the skin, helminth larvae must traverse the non-living stratum corneum, the cellular layers of the epidermis, and then the acellular barriers of the basement membrane and ground substance of the dermis. These acellular areas

have as their major constituent polysaccharide-containing protein which for convenience can be termed glycoprotein. If it is necessary for the parasite to enter the circulatory system or pass through other epithelia of the host it again encounters similar glycoprotein barriers surrounding the vessels or underneath the epithelium. The nature and characteristics of these acellular substances have been elucidated through a number of histochemical studies which show that in the mammalian host the degree of polymerization or aggregation of the polysaccharide-containing protein can be correlated with the intensity of staining by the Hotchkiss (Periodic Acid-Schiff) and Evans blue techniques (14, 15, 16). The basement membrane of the skin for example, in the adult host, is a thin, dense, highly polymerized, continuous sheet extending beneath the stratum germinativum. It forms a homogeneous matrix in which various fibrous elements may be embedded and it appears to have continuity with intercellular cement substances as well as with the less dense ground substance. Changes in the density of these glycoprotein elements with age or parturition and in disease processes are detected histochemically as a decrease or increase in staining intensity or loss of material with the Hotchkiss method or an increased or decreased binding of intravenously introduced Evans blue (14-17). Decreased density and loss of staining ability with the Hotchkiss method and increased binding of Evans blue is interpreted as indicating that the glycoprotein substances have depolymerized and that there is an increase in water-soluble components. A change of this type may be effected by collagenase enzymes. We have shown (18) that during skin penetration by *Schistosoma mansoni, Schistosomatium douthitti,* or *Strongyloides ratti,* alterations of the basement membrane and ground substance occur as shown by the Hotchkiss technique. They appear to soften or depolymerize. The basement membrane disappears prior to the actual entrance of the cercariae into the dermis and the ground substance becomes pale about the cercariae as they enter the dermis. In addition, as these larvae penetrate the skin, Evans blue is bound at the site of penetration as the acellular basement membranes and ground substance become more soluble. Similar but less extensive alteration of glycoprotein occurs about the eggs of schistosomes moving through tissues and at the site of penetration of the snail host by schistosome miracidia. Another parasite affecting acellular glycoprotein of tissue in the same manner is the hexacanth embryo of *Taenia saginata.* As it penetrates the intestinal mucosa (19) secretions of the larva alter intercellular cementing substances and also have some cytolytic effects. A further example of this type of activity is found in the developing larvae of *Taenia taeniaeformis* (20). During the first few days of its development in the liver of the rat, a period which is characterized by rapid growth

of the organism and displacement of hepatic cells, the developing strobilocercus causes marked changes indicative of enzymatic softening of the basement membranes and intercellular substances in its vicinity (20).

Other effects of tissue penetration that are to be noted in those forms that have demonstrable effects on host glycoprotein are changes in collagen and reticulin. Reticular fibers may be seen on occasion to be broken or to have lost their argyrophilia, and collagen fibers may become argyrophilic in connective tissue through which larvae are passing. In addition to evidence of enzymatic alteration of host glycoprotein other changes may be observed. After massive exposure of the skin to larvae of S. *mansoni* or S. *ratti* there is a decrease in protein-bound sulfhydryl groups of the dermis (21). This has been interpreted as a non-specific effect as it is probable that closely following the considerable alteration of material in this area to a more soluble state there is removal of the soluble products by the host circulation. Another effect not entirely related to the effect on glycoprotein is an increase in free water and an increased metachromosia about the penetration site. The latter two phenomena are common features in skin invasion by schistosomes and *Strongyloides,* both of which extensively alter the basement membrane and ground substance. However, they are also found following skin penetration by *Nippostrongylus muris* and tissue penetration by *Trichinella* neither of which exhibit histochemical evidence of enzymatic activity against the polysaccharide-containing protein. As mentioned previously, with peneration of the tissues there is usually a certain amount of cell destruction. However, in our experience and in the experience of others (4, 18, 19) cytolysis has not been an immediate prominent feature accompanying penetration of the tissues by individual helminths. The usual picture is one in which only one or two cells of the epithelium in the immediate vicinity of a larva are lysed; or a few cells may be freed and displaced.

It should be pointed out that in a number of those parasites which cause alteration of glycoprotein during penetration, e.g., the schistosomes and cestodes, the glands actively secreting at this time have as a major constituent a polysaccharide-protein substance. Prior to being secreted this is granular in appearance and when secreted may be deposited in strings or strands on the surface of the host epidermis or may be carried along with the pentrating larvae. In some instances secretions from glands of a similar nature in cercariae may coat the outer portions of the organism. It has been suggested that the secretion is an enzyme-polysaccharide complex (18-19) that by its viscous nature tends to localize the site of activity of the enzyme (18) or that it is a mucoid substance protecting the invading organism from the defense mechanisms of the host (23, 24).

The living eggs of schistosomes in tissues similarly have a glycoprotein secretion closely applied to their external surface (18, 22) and the miracidial penetration glands have a similar constituent. The presence of secreted polysaccharide about these organisms is extremely interesting when compared to the situation found in some invasive bacteria. In these the capsules are polysaccharide in nature and may contain some amino acid. The capsules function to protect the organism by inhibiting phagocytosis or digestion by host cells, and the introduction of capsular material into the host aids invasion by ordinarily noninvasive unencapsulated organisms. It is possible that the secretions play a dual role in serving to localize a readily diffusible enzyme and to protect the parasite from the host. It would be interesting to determine whether these polysaccharide substances are related to those shown by Oliver-Gonzalez (25) to be important antigen components in these infections.

The evidence of enzymatic activity of helminth larvae derived from histochemical studies is not precise since it does not elicit information on the exact nature of the enzymes altering the tissue components of the living host. However, the effects seen during these helminth invasions are remarkably similar to those resulting from the activity of certain bacterial toxins, particularly the collagenase-containing toxin of *Clostridium welchii A* (14, 16). Similar enzymatic activities and histochemical findings have been described in rapidly metatasizing tumors in locales where they are invading normal tissue (14, 16). More direct evidence that this similarity is more than superficial is available in the case of the cercariae of *S. mansoni* and *S. douthitti*, for the filariform larvae of *S. ratti* and for the early stages of development of the larvae of *T. taeniaeformis* (18). Most information exists for *S. mansoni* and *S. ratti*, and the following pertains primarily to these forms. Secretions of the living larvae are, like bacterial collagenase, capable of digesting thin gelatin films, and releasing dye from azo-dye-bound collagen substrates. Saline extracts of the larval homogenates are similarly active against these substrates, exhibit a general proteolytic activity against frozen dried tissue sections and also show extensive activity against the polysaccharide-containing protein of such sections. The activity of these saline extracts of helminth larvae in releasing dye from azotized hide-powder collagen and from azotized cartilage has provided a means of quantitative photometric comparison of the activities of different helminth extracts and also allows comparison to the activities of bacterial collagenase and proteolytic enzymes such as crystalline trypsin (21). From these tests it has been shown that the activity of the helminth-derived enzymes is directly proportional to the number of organisms in the test system or to the dry weight of organisms from which the aliquot of enzymes has been extracted. The activity

increases with time when incubated at 37°C and is destroyed by heating at 60°C. In contrast to the collagenase of *Cl. welchii* toxin, which is almost uniformly active over a relatively wide pH range, the enzyme of S. *mansoni* cercariae has a distinct optimum at pH 7.5 while that of S. *ratti* is in the vicinity of 7.0. The enzymes of the helminths and bacterial collagenase differ slightly in the degree of sensitivity to various metal ions. However, all were inhibited by mercury and copper and slightly activated by the presence of calcium and magnesium. Agents binding sulfhydryl groups inhibit the activity of the enzyme derived from S. *mansoni* but have little effect on that of S. *ratti*. The latter is more sensitive to chelating agents and sulfhydryl-containing compounds than is the cercarial enzyme. Both of these substances (S-H-binding and chelating) inhibit the activity of bacterial collagenase. In contrast to trypsin, and like the bacterial collagenase, both helminths' enzymes are active on cartilage and are not affected by the presence of the specific trypsin inhibitors. In comparison to bacterial collagenase, the activity of the helminth enzymes on native collagen of bovine origin *in vitro* is slight with only small amounts of hydroxyproline being released as amino acid or in polypeptides, although *in vivo* activity against collagen-containing tissues of the normal host is marked. The enzyme of S. *mansoni* cercariae, like trypsin, is capable of degrading urea-denatured hemoglobin to release tryosine, an activity which in the case of the helminth extract is not abolished by trypsin inhibitors. The substances inhibiting the helminth enzymes *in vitro*, when present at low concentrations, will in some instances retard or inhibit penetration. It is apparent from these studies that although there are points of resemblance, the enzymes derived from infective larvae of S. *mansoni* and S. *ratti* differ not only from each other but also from bacterial collagenase and trypsin. It should be emphasized again that this type of enzymatic activity is limited to those helminths which histochemically are shown to alter the polysaccharide-containing protein of the host and that this activity is absent in such effective tissue invaders as *Nippostrongylus muris* or *Trichinella spiralis*. Similarly these activities have not been found in extracts of various xiphidiocercariae or pharyngeate strigeid cercariae and only equivocal activity is found in extracts of filariform larvae of *Ancylostoma caninum*.

The parasite species possessing collagenase-like pentration enzymes do not exhibit this activity at all stages in their life histories. Thus, although proteolytic activity can be demonstrated in extract of adult schistosomes, this does not have the characteristics of the enzyme of the cercariae and is not inhibited by the same substances. Histochemical evidence suggests that the embryonated eggs and miracidia of the schistosomes secrete enzymes similar to those used by the cercariae during penetration (18).

Also, the possession of a collagenase-like activity is limited in *T. taeniaeformis* to the early, rapidly growing stage of the strobilocercus and is not found in older stages or in the adult tapeworm (20).

There is no direct information available on the presence of enzymes in other helminths that migrate extensively through connective tissue, but on a purely speculative basis it seems probable that collagenases or collagenase-like enzymes may be present in adult or developing-tissue stages of *Dracunculus, Loa, Onchocerca* and similar forms. Collagenases have, however, been reported from the highly invasive arthropod larvae, *Hypoderma bovis* (26), from the Australian sheep blowfly *Lucilia cuprina* (27), and from *Lucilia sericata* (28).

It has been convenient in the discussion so far to view the activities of those organisms which alter polysaccharide-containing protein during invasion as resulting from a single proteolytic enzyme. However, this is in all probability an oversimplification since the helminth penetration mechanism more probably is a complex system similar in this respect to bacterial toxins such as the toxin of *Cl. welchii* (29, 30). The most obviously useful functional enzyme to be sought for is a mucopolysaccharidase such as hyaluronidase. The discovery of hyaluronidase and its relationship to the invasiveness of bacteria has stimulated a number of investigators to search for a similar activity in animal parasites. A "spreading factor" has been described from larvae of *Ancylostoma duodenale* (31, 32), *Cercaria ocellata* and *Cercaria pseudarmata* (33) and cercariae of *Schistosoma mansoni* (34). In studies from our laboratory we have also described an effect somewhat similar to the "spreading" of hyaluronidase obtained following the intradermal injection of extracts of larvae of *S. mansoni, S. ratti, S. simiae, A. caninum,* and *N. muris,* as well as following the injection of extracts of non-invasive *Rhabditis pellio* (18). This response differs from that produced by hyaluronidase in that the "spreading" is usually accompanied by a swelling of the dermis and the production of a mucoid-appearing bleb that may be seen if the skin of the animal is viewed from the dermal surface. There was no correlation with this "spreading" and the effect of penetrating larvae on polysaccharide-containing protein of the skin.

Another indirect but sensitive method of demonstrating an activity of hyaluronidase, of undetermined specificity, is the streptococcal decapsulation test. This has been applied to the investigation of several parasites and a decapsulating factor has been reported for cercariae of *S. mansoni* (35, 36), and its presence suggested in *A. caninum* and *Entamoeba histolytica* (37). It has also been reported in *Balantidium coli* (38). In our own investigations (39), extracts of cercariae of *S. mansoni,* and filariform larvae of *S. ratti, A. caninum* and *N. muris* were found to be capable of

reducing the size of capsules of streptococci. The extracts of *S. mansoni* cercariae exhibited the greatest activity of those tested. None of the extracts in our preparations were capable of as complete a decapsulation of the bacteria as the hyaluronidase controls.

Our knowledge of the activity of enzymes of skin-penetrating helminths on more precisely characterized mucopolysaccharide substrates under well-controlled conditions is meager. A reduction in viscosity of hyaluronic acid incubated with cercariae of *S. mansoni* has been reported in one investigation in which the activity did not correlate directly with the number of cercariae (40).

Other investigators have been unable to demonstrate hyaluronidase in *S. mansoni* by similar methods (4). We have also been unable to demonstrate hyaluronidase activity in extracts of larvae of *N. muris, S. mansoni, S. ratti, S. simiae,* or *R. pellio* using a highly purified hyaluronic acid substrate derived from the streptococci whose capsules are reduced by these same extracts. In addition none of the helminth extracts tested exhibited activity toward chondroitin sulfuric acid or ovomucin. However, extracts of *S. mansoni* cercariae were found to be extremely active on one mucopolysaccharide substrate, heparin. It is further interesting in this connection that the toxin of *Cl. welchii* also is active against heparin but in addition has demonstrable activity against hyaluronic acid and ovomucin but not against chondroitin sulfuric acid.

The apparent disparity in the results of studies on the presence of mucopolysaccharidase activity in helminths may be due to a variety of factors. The methods that have been used vary greatly in their sensitivity. In addition, as with the "spreading factor" test, the methods used are not necessarily an index of a specific enzyme activity; for example, in some bacterial substances of high "spreading" potency no hyaluronidase is demonstrable (41). In many of the investigations the substrates utilized have been mixtures and the apparent activity measured may well be related to the degradation of substrate components other than hyaluronic acid. No functional significance can be ascribed at present to heparinase activity. The relatively high level of activity per organism in the case of *S. mansoni* suggests the possibility that if a more natural mucopolysaccharide substrate were used a significant level of activity might be found to which a functional interpretation might be ascribed. It is my feeling that at the present stage of our knowledge we can admit the presence of a mucopolysaccharidase activity but that the presence of hyaluronidase in the specific sense of the term is questionable. *In vivo,* hyaluronidase inhibitors do not affect the penetrating capacities of the parasites (21). The finding that mucopolysaccharidase activity is present at a low level in a variety of tissues and undifferentiated epithelia suggests the possi-

bility that the low levels that may be present in these helminth extracts is related to a function other than penetration.

The only remaining enzymatic activity that has been definitely identified in helminth larvae for which a function in invasion has been suggested is the lipase of *Nippostrongylus* described by Thorson (42). He has demonstrated that secretions and extracts of infective larvae are capable of producing fatty acids from a vegetable fat substrate. We have recently demonstrated a lipase capable of acting in a similar manner on tripalmitin, a normal skin constituent, in extracts of filariform larvae of *Nippostrongylus muris* and *Strongyloides ratti*, and in extracts of cercariae of *Schistosoma mansoni* (43). At present the significance of these findings is not clear and we hesitate to place a functional interpretation on their presence.

Up to this point of my discussion we have been concerned with the observed or measured effects of the parasite on certain of the normal host substrates that are encountered during initial invasion or passage through tissues. During this process the parasite is exposed to a multitude of factors and host substances any one of which may alter the ultimate success of the invading individual. One of these we have not previously mentioned is the state of the stratum corneum. The relative success of the invading individual has been shown by Stirewalt (44) to be influenced by its integrity and the nature of the surface. I should like to emphasize that, excluding the other factors, the physical or physiological state of one of these substrates, the host glycoprotein, may be of great significance to the relative success of the invading parasite. It is a barrier to penetration that fluctuates in its effectiveness and in its physical integrity with the age or physiological state of the host (14, 17). The basement membranes and ground substance of young animals are diffuse and nonresistant, allowing skin-penetrating helminths to traverse them with ease and rapidity. In older animals these are thicker and denser and more resistant to enzymatic alteration. Consequently not only is penetration slower in older animals but also a relatively high percentage of the larvae are retained in the skin, excluded from the dermis by the unaltered basement membrane. Hypophysectomized young rats develop highly polymerized basement membranes in the skin and resemble aged animals in this respect. When speed and relative success of penetration of these are compared with those of normal litter mates the hypophysectomized animals exhibit a condition analogous to that of the older animals in that larvae take longer to penetrate and many are not readily able to alter the basement membrane and gain access to the dermis. This suggests that the age immunity operative in some parasitic infections has non-humoral aspects and may develop in the absence of natural antibody or an antibody

response engendered by previous exposure to homologous or related antigens. It also suggests the possibility that the absolute or relative immunity of a species to particular skin-penetrating parasites might be related to the constitution of this glycoprotein barrier. It is possible that slight species differences in the chemical nature of the barrier would make it partially or completely resistant to the parasite enzyme system. This concept might explain the usually superficial nature of cutaneous larval migrans as discussed by Beaver (45) and the observations that traumatic damage to the skin is in some instances followed by penetration and deeper migration of canine hookworm in man (46, 47). It is interesting in this respect that the larvae of *Ancylostoma caninum* have realtively weak enzymatic activity against glycoprotein when compared to that of the schistosomes and *Strongyloides* (21). These cases of age and species immunity might then be considered a special type of athreptic immunity, as they would be dependent on the presence or state of substrate that cannot be satisfactorily altered by the parasite enzymes.

Certain other conditions of the host such as male castration, cryptorchidism, or adrenalectomy also increase the density of the basement membranes (14, 16) and might also increase the resistance of the host to invasion. Although there is little experimental evidence utilizing this type of host in parasitic infections it has been shown that castrated males' resistance to infection by *Taenia taeniaeformis* is increased (48).

Conversely, it is interesting to speculate, even in the absence of completely sufficient experimental evidence, on what may happen if the glycoprotein barriers of the host are changed by normal physiologic or pathologic processes which alter glycoprotein elements of connective tissue in the direction of decreased density and resistance.

In certain normal and pathological conditions, the basement membranes and ground substance become more plastic and less highly polymerized and might presumably more readily be invaded by parasites. Scorbutic animals, for example, have notably depleted, spongy, altered glycoprotein and are known to be more susceptible to invasions including those of pathogenic amebae (49). However, we are without experimental information as to their reaction to parasitic skin-penetrating helminths. Similarly, the lowered resistance of pregnant hosts to helminth invasions reported by various investigators (50, 51, 52) may possibly be correlated with the greater plasticity and lessened resistance of host glycoprotein substances that develops during this time.

This avenue of speculation also allows us to present a rational hypothesis in explanation of some of the perplexing results obtained in parasitic infections of animals treated with various gonadal hormones. For example, it has been shown by Sadun (53, 54) and by Ackert and Dewhirst (55)

that when moderate doses of testosterone, alphaestradiol or diethylstilbestrol are administered to immature male or female chickens, there is an increase in the resistance to infection by *Ascaridia*. This may possibly be ascribed to the earlier maturation of the connective tissue barriers such as the basement membranes which occurs under the influence of the moderate hormone dosage. On the other hand, Sadun (53), has also shown that when heavy doses of testosterone are administered there is a marked decrease in resistance of the host, with approximately four times as many individuals successfully completing the invasion of the host, This may also be related to the state of the sub-epithelial glycoprotein, as following large doses of testosterone or estradiol there is a striking decrease in this natural barrier.

In each of the instances of hormonal influence on invasiveness that have been cited it is obvious that there are other profound physiological changes in the host in addition to the decreased or increased resistance of the connective tissue glycoprotein. For example, important factors also bearing on invasiveness are the direct effect of gonadal hormones on growth and maturation of parasites, and alteration of antibody response. It is possible that since massive doses of cortisone reduce the glycoprotein barrier (14, 16, 56) this is closely related to the enhancement of parasitism by certain helminths (57, 58) following its administration. However, in this instance we must include in our explanation of its effects the important simultaneous inhibition of inflammatory response and the involution of lymphoid tissue and its effects on antibody production.

The final aspect of invasiveness of the host by helminth larvae that I should like to present deals with one limited aspect of the humoral responses of the host to tissue penetration. It is general knowledge that the immune host is able to inhibit the passage of larvae through its tissues, immobilizing the parasites as they pass through the skin or lungs, precipitating their secretions and metabolic products, and ultimately encapsulating and destroying many of the invading individuals. These phenomena have been the subject of many papers and reviews. It has been suggested that specific anti-enzyme antibodies are produced by the immune host; these would include antibodies acting against the penetration enzymes of helminth larvae (59, 60). The direct demonstration of this is not well documented in the case of invading helminth larvae in actively immunized animals. Thorson has shown that the lipase of *Nippostrongylus muris* is inhibited by serum of animals immunized by previous infection and not by normal serums (42). He has further shown that the proteolytic enzymes of *Ancylostoma* are antigenic and that antiserums may be produced that inhibit the enzyme and suppress the infection (61). The effects of various serums on the collagenase-like penetration enzymes

of S. *mansoni* cercariae and of *Strongyloides ratti* larvae have also been studied and compared to the effects on the collagenase-containing *Cl. welchii* toxin and to trypsin (62). Normal serums from a variety of animals at 1/1000 dilution will completely inhibit trypsin even after being heated to 56°C for 20 minutes. A comparable amount of bacterial toxin (calculated as dye released from azocoll) is either unaffected or activated by this treatment. From 50 to 80% of the activity of the equivalent amount of enzyme of S. *mansoni* is lost in the presence of unheated normal serums of various sources at this dilution. Normal human serum heated at 56°C for 20 minutes does not inhibit the cercarial enzyme. However, in active human infection with S. *mansoni* in a series of 55 individuals at 1/1000 dilution of heat-treated serum, there was an average inhibition of enzyme activity of approximately 40%, with serum of some individuals reducing the activity as much as 70%. It was impossible to correlate the possession of a high level of cercarial enzyme inhibitor in infected individuals with age, sex, therapy received, or clinical status of the infection except that the inhibitor was present in individuals with presently or recently active infections. The 10 individuals of the group showing the most strongly positive skin reactions to cercarial antigen exhibited no greater inhibition to cercarial enzyme than did the whole group. In order to determine whether the anti-collagenase activity of serum from infected individuals was related to the serum's other activities against schistosome antigens, selected serums were separated by starch electrophoresis. The various fractions were tested for their ability to agglutinate cercariae, to agglutinate miracidia, to cause circumoval precipitates, for their complement fixing activity, and for their ability to inhibit trypsin (63). It was determined that the inhibitor of the cercarial enzyme migrates in the region of the alpha globulin and is more sharply limited in its distribution than is the serum trypsin inhibitor. The complement fixing antibodies were found to parallel the distribution of the gamma 2 globulins; the cercarial agglutinin is also within the gamma globulin area and rather sharply limited in its distribution. The miracidial agglutinin, although more widely distributed than the circumoval precipitin, has its peak activity in the same fractions of the gamma 1 globulins. Of the several serums tested following starch electrophoresis, all of those having high levels of anti-collagenase activity have also exhibited circumoval precipitins and miracidial agglutinins. Some of these were lacking in perceptible complement-fixing antibody or cercarial agglutinin although a few, including normal serums, had a factor immobilizing or killing cercariae, that migrated even more rapidly than the collagenase inhibitor or albumins. The enzyme inhibitor is storage labile but not nearly so labile as the cercaricidal substances of normal serum described by Culbertson (61). No precipitates are formed when the inhibitor is incubated with cercarial

extracts. Although it gradually disappears from frozen serum over a period of several months it is stable for months after separation from serum by starch electrophoresis. Its activity is lost if the serum is treated with protein precipitants.

Although increased levels of the collagenase inhibitor are found in serums from infected individuals there is no assurance that this is an antibody phenomenon. In case of normal serums from man, mouse and the rabbit, heating of the serum abolishes the inhibition, whereas in those from the monkey, guinea pig and rat the antienzymatic activity is lowered but not abolished. Rats immunized by repeated exposure to *S. ratti* do not have demonstrably higher titers of collagenase-inhibiting substances than do normal animals. Nor do immunized monkeys or mice with active infections have titers against the collagenase of *S. mansoni* that are demonstrably higher than in the normal animal. Antibody has in at least one instance been shown to migrate with the alpha globulin fraction of serum following immunization with antigens derived from the tubercle bacillus (65). In addition this serum fraction may contain C-reactive protein, various glycoproteins including orosomucoid, and inhibitors of hyaluronidase, trypsin, plasmin and hemagglutinin of influenza virus (66, 67, 68). These may increase during infection or following tissue destruction. It has been determined that the inhibitor of the collagenase of *S. mansoni* cercariae is not identical to any of these substances and is not increased in various bacterial infections, rheumatic fever or acromegaly.

In summary, our knowledge and understanding of the precise mechanisms utilized by helminths in penetration of the tissues of the host is very incomplete. Some forms, e.g., *Schistosoma, Strongyloides,* have been shown to possess enzymes that are proteolytic and collagenase-like in nature. These species cause extensive enzymatic alteration of the acellular, glycoprotein, connective tissue barriers of the host as they penetrate. In addition, the same species may possess mucopolysaccharidases and lipases. Other effective tissue-penetrating larvae such as *Nippostrongylus* are lacking in collagenase-like enzymes and are not known to possess mucopolysaccharidase, but do have lipolytic substances. The penetration enzymes are not present at all stages of growth and development of the parasite but in certain forms at least are limited to those stages which actively penetrate or displace host tissues.

From these investigations it is apparent that in addition to the better known humoral and cellular immune responses, another element in resistance of the host to invasion must be considered. This is the state of organization of the acellular glycoprotein that forms the intercellular cement, subepithelial basement membranes, and ground substance of the host. These substances have been shown to vary in their density and

resistance to enzymatic alteration with the age of the host, nutritional status and hormonal influences. These variations correlate in many instance with the relative susceptibility or resistance to invasion that the host displays. It is suggested that this is a major factor in accounting for the increased resistance with age of the host, and the decreased or increased resistance exhibited by hosts under normal or induced gonadal-hormone changes. Species immunity as well may in part be related to the state of these glycoprotein substances, as it is conceivable that species differences in composition might make these more effective barriers with respect to the enzymes of a particular parasite.

Although there is an abundance of evidence that parasite secretions and products cause the production of precipitating antibodies, specific evidence that humoral responses are directed against the parasite penetration enzymes is meager. *Nippostrongylus* lipase is inhibited by serum of animals actively immunized with this species; but the role of lipase in penetration is speculative. The anticollagenase titer is markedly increased in some human infections with schistosomes. Since a lower titer of similar substance is normally present and since the antienzyme is associated with the alpha globulin serum fraction it is at present inappropriate to consider it an antibody. It varies in its amount and effectiveness from species to species and may function in resistance, possibly playing a role in species immunity. In human infection it is possible that, as suggested by Newsome (69), the maturation of the infection may cause release from the host of some non-antibody substance, such as this, which is inimical to developing or migrating worms.

In conclusion, I should like to emphasize that our knowledge of the mechanisms involved in the penetration of host tissues and cells by animal parasites is still relatively superficial, and that our knowledge of the natural or acquired defenses of the host as related to these mechanisms is similarly deficient. It is to be hoped that future investigations will result in the isolation and precise characterization of the enzyme-substrate systems involved not only in helminth invasions but also in the cellular and tissue invasions of protozoa. The information resulting from such studies should amplify our knowledge of the specific immune responses of the host and should also result in a rational concept of the nature of natural immunity and resistance and the reasons for their variability.

REFERENCES

(1) Taliaferro, W. H. 1955. Specificity in the relationship between host and animal parasites. *Biological Specificity and Growth,* Princeton University Press, Princeton, New Jersey. 157-176.

(2) Vogel, H. 1932. Hauterscheinungen bei Schistosomiasis: Beobachtungen über Zerkarien-Dermatitis, Kutanreactionen und ein Vulva-Granulom. *Arch. f. Schiffs- u. Tropen-Hyg.* 36, 384-399.

(3) Pinto, C., and De Almeida, F. A. 1945. Penatração das cercarias de *Schistosoma mansoni* na pele de *Canis familiaris* e do homen. *Rev. brasil. biol.* **5**, 219-229.
(4) Gordon, R. M., and Griffiths, R. B. 1951. Observations on the means by which the cercariae of *Schistosoma mansoni* penetrate mammalian skin, together with an account of certain morphological changes observed in the newly penetrated larvae. *Ann. Trop. Med.* **45**, 227-243.
(5) Griffiths, R. B. 1953. Further observations on the penetration of mammalian skin by the cercariae of *Schistosoma mansoni*, with special reference to the effects of mass invasion. *Ann. Trop. Med.* **47**, 86-94.
(6) Girges, R. 1934. *Schistosomiasis (Bilharziasis)*, John Bale and Sons, London.
(7) Faust, E. C. 1946. The diagnosis of *Schistosomiasis japonica*. II. The diagnostic characteristics of the eggs of the etiologic agent *Schistosoma japonicum*. *Am. J. Trop. Med.* **26**, 113-123.
(8) Hoeppli, R. 1932. Histological observations in experimental *Schistosomiasis japonica*. *China M. J.* **46**, 1179-1186.
(9) Standen, O. D. 1952. The penetration of *Schistosoma mansoni* into the skin and lymphatics of the mouse. *Tr. Roy. Soc. Trop. Med. & Hyg.* **46**, 384.
(10) Jensen, V., and Roth, H. 1938. Zur Einwanderung der Trichinenlarvae in die quergestreifte Muskelfaser. *Acta pathol. et microbiol. Scandinav. Suppl.* **37**, 259-271.
(11) Hoeppli, R. 1933. On histolytic changes and extraintestinal digestion in parasitic infections. *Lingnan Sc. J.* **12**, 1-11.
(12) Davis, D. J. 1936. Report on the preparation of an histolytic ferment present in the bodies of cercariae. *J. Parasitol.* **22**, 108.
(13) Hunter, G. W., and Hunter, W. S. 1937. Studies on host relations to larval parasites. III. An histolytic ferment from the cercariae of *Cryptocotyle lingua*. *J. Parasitol.* **23**, Suppl., 572.
(14) Gersh, I., and Catchpole, H. R. 1949. The organization of ground substance and basement membrane and its significance in tissue injury, disease and growth. *Am. J. Anat.* **85**, 457-522.
(15) Gersh, I. 1950. Glycoproteins in the thyroid gland of rats. *J. Endocrinol.* **6**, 282-287.
(16) Gersh, I. 1952. Ground substance and the plasticity of connective tissues. *The Harvey Lectures*, The Harvey Society of New York. Charles C Thomas, Springfield, Illinois. 211-241.
(17) Perl, E., and Catchpole, H. R. 1950. Changes induced in the connective tissue of the pubic symphysis of the guinea pig with estrogen and relaxin. *Arch. Pathol.* **50**, 233-239.
(18) Lewert, R. M., and Lee, C. L. 1954. Studies on the passage of helminth larvae through host tissues: I. Histochemical studies on extracellular changes caused by penetrating larvae. II. Enzymatic activity of larvae *in vitro* and *in vivo*. *J. Inf. Dis.* **95**, 18-51.
(19) Silverman, P. H., and Maneely, R. B. 1955. Studies on the biology of some tapeworms of the genus *Taenia*. III. The role of the secreting gland of the hexacanth embryo in the penetration of the intestinal mucosa of the intermediate host, and some of its histochemical reactions. *Ann. Trop. Med. and Parasitol.* **49**, 26-330.
(20) Lewert, R. M., and Lee, C. L. 1955. Studies on the passage of helminth larvae through host tissues. III. The effects of *Taenia taeniaeformis* on the rat liver as shown by histochemical techniques. *J. Inf. Dis.* **97**, 177-186.
(21) Lewert, R. M., and Lee, C. L. 1956. Quantitative studies of the collagenase-like enzymes of cercariae of *Schistosoma mansoni* and *Strongyloides ratti*. *J. Inf. Dis.* **99**, 1-14.
(22) Katz, F. F., and Carrera, G. M. 1957. The reaction of *Schistosoma mansoni* egg shells to the periodic acid-Schiff staining procedure. *J. Parasitol.* **43**, 24.
(23) Kruidenier, F. J. 1953. Studies on mucoid secretion and function in the cercaria

of *Paragonimus kellicotti* Ward (Trematoda: Troglotrematidae. *J. Morphol.* **92**, 531-538.
(24) Kruidenier, F. J., and Stirewalt, M. A. 1954. Mucoid secretion by schistosome cercariae. *J. Parasitol.* **40**, Suppl. 33.
(25) Oliver-Gonzalez, J. 1955. Immunological properties of polysaccharides from animal parasites. *Ann. Rev. Microbiol.* **9**, 353-362.
(26) Lienert, E., and Thorsell, W. 1955. Untersuchungen über die Activität von Autolysaten aus Wanderlarven (Hypoderma bovis) auf elemente des Bindgewebes. *Exp. Parasitol.* **4**, 117-122.
(27) Waterhouse, D. F., and Irzykiewicz, H. 1957. An examination of proteolytic enzymes from several insects for collagenase activity. *J. Insect Physiol.* **1**, 18-22.
(28) Hobson, R. P. 1931. An enzyme from blow-fly larvae (*Lucilia sericata*) which digest collagen in alkaline solution. *Biochem. J.* **25**, 1458-1463.
(29) Oakley, C. L., Warrack, G. H., and Van Heyningen, W. E. 1946. The collagenase (k-toxin) of *Cl. welchii* type A. *J. Pathol. & Bacteriol.* **58**, 229-253.
(30) Bidwell, E., and Van Heyningen, W. E. 1948. The k-toxin (collagenase) of *Colostridium welchii. Biochem. J.* **42**, 140-151.
(31) Bruni, A. 1939. Il fattore de diffusion nell *Ankylostoma duodenale. Settimana Med.* **17**, 1105-1106.
(32) Bruni, A., and Passalacqua, A. 1954. Sulla presenza di una mesomucinase (jaluronidasi) in *Ancylostoma duodenale. Boll. Soc. Ital. Sper.* **30**, 789-791.
(33) Ginetsinkaya, T. A. 1950. New data on the mechanism of penetration and migration of cercariae in the tissue of the host. *Dokladi Academi Nauk SSSR* **72**, 433-435.
(34) Kuntz, R. E. 1953. Demonstration of the "spreading factor" in the cercariae of *Schistosoma mansoni. Exp. Parasitol.* **2**, 397-402.
(35) Stirewalt, M. A., and Evans, A. S. 1952. Demonstration of an enzymatic factor in cercariae of *Schistosoma mansoni* by the streptococcal decapsulation test. *J. Inf. Dis.* **91**, 191-197.
(36) Evans, A. S. 1953. Quantitative demonstration of hyaluronidase activity in cercariae of *Schistosoma mansoni* by the streptococcal decapsulation test. *Exp. Parasitol.* **2**, 417-427.
(37) Lincicome, D. R. 1953. A streptococcal decapsulation test for detection of hyaluronidase activity in animal parasites. *Exp. Parasitol.* **2**, 333-340.
(38) Tempelis, C. H., and Lysenko, M. G. 1957. The production of hyaluronidase by *Balantidium coli. Exp. Parasitol.* **6**, 31-36.
(39) Lee, C. L., and Lewert, R. M. 1957. Studies on the presence of mucopolysaccharidase in penetrating helminth larvae. *J. Inf. Dis.* **101**, 287-294.
(40) Levine, M. D., Garzoli, R. F., Kuntz, R. E., and Killough, J. 1948. On the demonstration of hyaluronidase in cercariae of *Schistosoma mansoni. J. Parasitol.* **34**, 158-161.
(41) Meyer, K., Chaffee, E., Hobby, G., and Dawson, M. 1941. Hyaluronidases of bacterial and animal origin. *J. Exp. Med.* **73**, 309-326.
(42) Thorson, R. E. 1953. Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris. Am. J. Hyg.* **58**, 1-15.
(43) Lewert, R. M., and Mandlowitz, S. Unpublished data.
(44) Stirewalt, M. A. 1956. Penetration of host skin by cercariae of *Schistosoma mansoni.* I. Observed entry into skin of mouse, hamster, rat, monkey and man. *J. Parasitol.* **42**, 565-580.
(45) Beaver, P. C. 1956. Larva migrans. *Exp. Parasitol.* **5**, 587-621.
(46) Ellenbogen, V. 1930. Experimenteller Beiträg sur Frage der durch die Larven von *Ancylostoma caninum* beim Menschen verursachten Hauterscheiningen. *Klin. Wochenschr.* **9**, 1583-1585.
(47) Fülleborn, F. 1927. Durch Hakenwurmlarven des Hundes (*Uncinaria stenocephala)* beim Menschen erzeugte "creeping eruption." *Abhandl. geb. Auslandsk.* **26**, 121-133.

(48) Campbell, D. H., and Melcher, L. R. 1940. Relationship of sex factors to resistance against *Cysticercus crassicollis* in rats. *J. Inf. Dis.* **66**, 184-188.
(49) Sadun, E. H. 1950. The effect of dietary ascorbic acid deficiency on the susceptibility of guinea pigs to infection with *Endamoeba histolytica*. *J. Parasitol.* **36**, Suppl. 21.
(50) Wickramasuriya, G. A. W. 1937. *Malaria and ancylostomiasis in the pregnant woman.* Humphrey Wilford, London.
(51) Stoll, N. R. 1940. Worm-host systems as labile mechanisms: a view of the nematode-ruminant problem. *J. Am. Vet. Med. Ass.* **96**, 305-308.
(52) Whitlock, S. C. 1937. An apparent case of sexual difference in resistance to parasitic infection. *J. Parasitol.* **23**, 426.
(53) Sadun, E. H. 1948. Relation of the gonadal hormones to the natural resistance of chickens and to the growth of the nematode *Ascaridia galli*. *J. Parasitol.* **34**, Suppl. 18.
(54) Sadun, E. H. 1951. Gonadal hormones in experimental *Ascaridia galli* infection in chickens. *Exp. Parasitol.* **1**, 70-82.
(55) Ackert, J. E., and Dewhirst, L. W. 1950. Resistance of fowls to parasitism affected by female sex hormones. *J. Parasitol.* **24**, Suppl. 13-14.
(56) Seifter, J., Ehrich, W. E., Baeder, P. H., Britt, A. J., and Hauser, E. A. 1953. Evidence for the direct effect of steroids on the ground substance. In Mechanism of corticosteroid action in disease processes. *Ann New York Acad. Sci.* **56**, 623-814.
(57) Coker, C. M. 1956. Some effects of cortisone in mice with acquired immunity to *Trichinella spiralis*. *J. Inf. Dis.* **98**, 39-44.
(58) Coker, C. M. 1955. Effects of cortisone on *Trichinella spiralis* infections in non-immunized mice. *J. Parasitol.* **41**, 498-504.
(59) Chandler, A. C. 1932. Susceptibility and resistance to helminth infections. *J. Parasitol.* **18**, 135-152.
(60) Chandler, A. C. 1936. Studies on the nature of immunity to intestinal heminths. III. Renewal of growth and egg production in *Nippostrongylus* after transfer from immune to non-immune rats. *Am J. Hyg.* **23**, 46-54.
(61) Thorson, R. E. 1956. Proteolytic activity in extracts of the esophagus of adults of *Ancylostoma caninum* and the effect of immune serum on the activity. *J. Parasitol.* **42**, 21-25.
(62) Lee, C. L., and Lewert, R. M. Studies on the serum inhibitor of collagenase-like enzymes of *S. mansoni* cercariae. Manuscript in preparation.
(63) Lewert, R. M., and Lee, C. L. The distribution of various reactants in human anti-*Schistosoma mansoni* serum fractionated by starch electrophoresis. Manuscript in preparation.
(64) Culbertson, J. T. 1936. The cercaricidal action of normal serums. *J. Parasitol.* **22**, 111-125.
(65) Cole, E. R., and Favour, C. B. 1955. Correlations between plasma protein fractions, antibody titers and the passive transfer of delayed and immediate cutaneous reactivity to tuberculin PPD and tuberculopolysaccharides. *J. Exper. Med.* **101**, 391-420.
(66) Jacobson, K. 1955. Studies on fibrinogen. II. Studies of the trypsin and plasmin inhibitors in human blood serum. *Scand. J. Clin. Lab. Inv.* **14**, 57-102.
(67) Tyrrell, D. A. J. 1954. Separation of inhibitors of hemagglutination and specific antibodies for influenza viruses by starch zone electrophoresis. *J. Immunol.* **72**, 494-502.
(68) Weiner, A. E., Mehl, J. W., and Winzler, R. J. 1950. Studies on the mucoproteins of human plasma. V. Isolation and characterization of a homogeneous mucoprotein. *J. Biol. Chem.* **185**, 561-568.
(69) Newsome, J. 1956. Problems of fluke immunity: with special reference to schistosomiasis. *Trans. Roy. Trop. Med. & Hyg.* **50**, 258-274.

THE SYNTHESIS AND ACTIVITIES OF ANTIBODIES

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THIS evening I should like to consider the growing evidence that antibodies formed in a given species may differ widely in physicochemical properties and that these differences may be associated with marked differences in biological action. These findings, I believe, are of importance both to parasitologists interested in the action of antibodies on parasites and to other biologists using antibodies as experimental tools. The same material also forms an appropriate introduction to a discussion of the synthesis of antibody. The two subjects are so broad that I shall limit myself to a few illustrations, most of which are drawn from the work of the group associated with Dr. D. W. Talmage in the Department of Medicine of the University of Chicago and the group associated with Mrs. Taliaferro and me.†

Antibody has always been defined primarily in terms of its ability to combine with the antigen which induced its formation or with closely related substances and, secondarily, with its origin or increase following immunization. It was early recognized that most antigen-antibody reactions involve two stages: the first or primary rapid union of antigen and antibody and the second or visible stage, such as precipitation, lysis and biological neutralization. The so-called unitarian hypothesis gave a much needed simplification to the concept of antigen-antibody reactions. It led to the idea that the primary stage is fundamentally the same no matter what the secondary manifestation. Whether the latter is precipitation, lysis, etc., depends not on the nature of the antibody but on the physical state of the antigen, on environmental conditions, such as pH and salt concentration and, in some cases, on cooperative agents, such as phagocytes and complement (see Zinsser, 1931).

Most classic methods of measuring antibody have made use of the variable secondary stage. At the height of the reign of the unitarian hypothesis, this procedure offered little cause for concern since each

* Most of the author's work cited was done in part under contract AT(11-1)-175 between the U. S. Atomic Energy Commission and the University of Chicago and in part with grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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antibody could presumably show any secondary stage provided the necessary conditions were satisfied. Evidence is accumulating, however, that antibodies produced in the same animal with the same specificity may vary in their ability to trigger or support the visible secondary stage. A well known early example of this is nonprecipitating antibody. Recently, increased attention has been paid to the primary union of antigen and antibody as measured by the antigen-combining capacity of antibody (review by Talmage, 1957a). Here, also, the physico-chemical properties of antibody are important.

I should like to illustrate some of the complexities of the antigen-antibody reactions by discussing certain aspects of the secondary immunological phenomena of precipitation, skin sensitization, lysis and ablastic action. These examples will bring out the difficulties in measuring the *total* amount of antibody in the serum and in definitively characterizing antibody. These are the phenomena with which you, as biologists, are particularly concerned. This point needs emphasis since biologists and clinicians will and should be most interested in the biological effects produced by the secondary stage of immunological reactions.

The secondary reaction of precipitation has become the central immunological reaction of the immunochemists. It is the simplest one since both reactants are in solution and do not need complex accessory agents. Even more important, exquisitely fine methods of weighing antibody have been developed, notably by Heidelberger and his coworkers. Nevertheless, the so-called quantitative precipitin test has drawbacks for biological workers. It does not necessarily measure the biological activity of antibody or the total amount of antibody since it selects antibody having special properties including a relatively high avidity and a low rate of dissociation.

An excellent example of the limited nature of the quantitative precipitin test is shown by Farr (1956) for the amount of antigen bound and precipitated by antibody in the P_{80} test and in the antibody-binding capacity (= ABC) test.* The P_{80} test of Talmage and Maurer (1953) determines the amount of precipitate at the point of 80% antigen precipitation and gives results closely similar to the quantitative precipitin test (cf. W. H. and L. G. Taliaferro, 1957). The antigen-binding capacity* is determined by the gamma ray counts of I^{131}-labeled BSA (bovine serum albumen) in precipitate when mixtures of varying amounts of I^{131}-labeled BSA and constant amounts of unlabeled antiBSA serum are mixed in 50% saturated ammonium sulfate. Under these conditions, the uncombined I^{131}-labeled BSA, being an albumin, remains in solution while the I^{131}-labeled BSA bound to antiBSA is precipitated along with

* In this test, the antigen has to be soluble in half saturated ammonium sulfate.

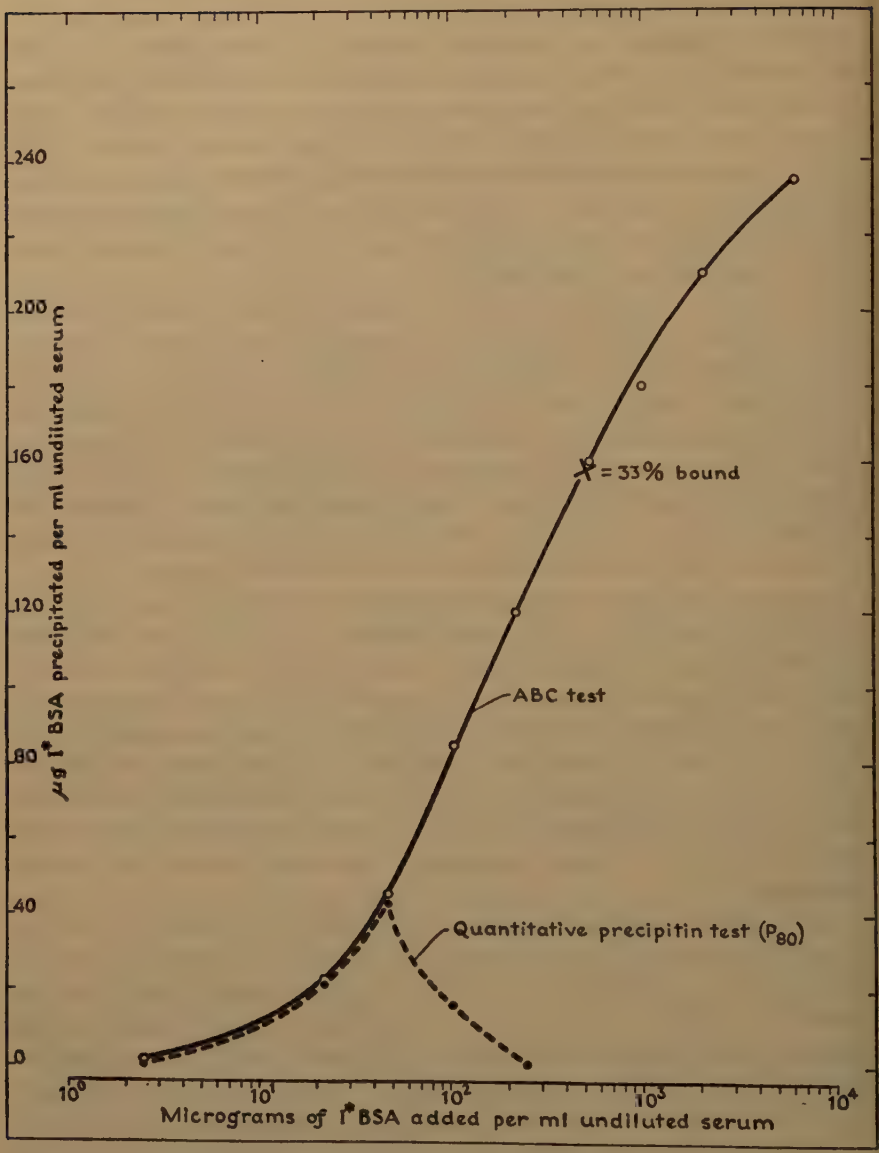


FIG. 1. The amount of antigen precipitated with antibody as measured by the quantitative precipitin (= P_{80}) test and the antibody-binding capacity (= ABC) test when increasing amounts of I^{131}-labeled BSA are added to a specific antiserum. (From unpublished data by Dr. R. S. Farr.) Note that per ml serum both tests registered practically the same amount of precipitable antigen up to 40 μg of I* BSA, but thereafter the tests registered markedly different amounts as additional antigen was added.

unlabeled antibody and other serum globulins. With the antiserum illustrated in figure 1, the maximum amount of antigen (40 μg. of BSA N) was precipitated in the P_{80} test by using 50 μg. of BSA nitrogen per ml. of antiBSA. Less antigen was precipitated when larger quantities of antigen were added. In marked contrast, the amount of antigen bound and precipitated increased as more and more antigen was added in the ABC test. At the highest point of the curve, 238 μg. of BSA nitrogen were precipitated by using 6000 μg. of BSA nitrogen per ml. of antiBSA. Farr further found that the ratio of antigen bound and precipitated in the two tests as well as the avidity vary widely among different serums.

Antibody acting as a precipitin has been of especial interest to parasitologists since the work of Blacklock, Gordon and Fine (1930) on fly larvae. Similar studies were made on various helminths by Sarles and Taliaferro (1936), Sarles (1938), Taliaferro and Sarles (1939 and 1942), and many subsequent workers. The reports indicate that precipitates may play a role in immunity to certain of the metazoan parasites. The formation of visible precipitates in and around fly larvae and worms is striking, but we may have gone too far in picturing the antibody effects in terms of this immune precipitate. As previously pointed out (Taliaferro, 1943), experiments on passive transfer are conclusive in showing that the prevention of feeding, stunting, immobilization, occasional death and expulsion of *Nippostrongylus muris* from the intestine are antibody effects, but the antibody need not necessarily act as a precipitin (i.e., be precipitating).

Kuhns and Pappenheimer (1952 a and b) and Kuhns (1954) have studied the biological antitoxic activity of nonprecipitating diphtheria toxin. They have clearly demonstrated the formation of two types of antitoxin in most persons immunized with diphtheria toxoid. The classical type is precipitating, is not skin-sensitizing since it leaves an injected site in about 100 minutes, and fixes complement in high titer. The nonprecipitating type is skin-sensitizing to toxin and toxoid since it remains at the site of injection for weeks. It fixes complement only slightly and loses its skin-sensitizing property after heating at 56°C for 4 hours. Both antibodies can passively sensitize guinea pigs to fatal anaphylactic shock. Briefly, therefore, the first type is a typical precipitating antibody, whereas the second one is indistinguishable from the heat labile, skin-sensitizing atopic antibody of asthma and hay fever. In spite of these differences, the nonprecipitating antibody is a highly effective antitoxin in neutralizing toxin in vivo. The nonprecipitating antibody is electrophoretically localized in the fast moving globulins (γ_1 or T fraction) and the precipitating antitoxin remains in the slow-moving globulins (γ_2 fraction). As my talk develops, we shall see that a number of biologically active antibodies belong to the fast moving or γ_1 globulins. The non-

precipitating antibody coprecipitates with precipitating antibody in the same way that nonprecipitating antibody frequently formed by rabbit does. Later, Kuhns (1955) found a second nonprecipitating antibody which diffuses rapidly from the site of inoculation and does not therefore sensitize the skin.

When one contemplates the implications of the various precipitating and nonprecipitating antibodies, the problem of accurate characterization is fascinating but certainly complicated. The situation would be somewhat simplified if each type of antitoxin, for example, had a different specificity and could neutralize the same toxin by combining with it at different sites or if the precipitating antitoxin combined with toxin or toxoid and the skin-sensitizing antitoxin combined with a tissue cell-toxin compound (review by Talmage et al, 1956). The possibility of an antitoxin to a tissue cell-toxin compound is suggested by the work of Ackroyd (1949) who demonstrated a sensitizing antibody to a sedormid (allylisopropylacetylurea)-platelet complex. Kuhns and Pappenheimer, however, believe that the ability of the precipitating and nonprecipitating antitoxins to neutralize the toxin is evidence of their identical specificity. Here, nevertheless, as in the later discussion of hemolysins, it is clear that there is no absolute method of determining specificity because two antibodies may combine with different sites on the antigen but may still be close enough to block each other partially or completely.

In many worm infections, a skin-sensitizing antibody is common. In 1928, Coventry and I found that 73% of 90 Honduraneans who were either currently infected or probably previously infected with ascaris gave the immediate wheal type of skin reaction after a scratch test with low concentrations of ascaris antigen, whereas the serums of 60% elicited antiascaris precipitins, 43% gave evidence of both antibodies and 11% gave neither. This scratch test with low concentrations of antigen is characteristic of the skin-sensitizing nonprecipitating antibody. The possible role of nonprecipitating and skin-sensitizing antibodies in worm infections seems to me one of the most intriguing problems in parasitic immunity.

Another pertinent illustration of the diversity of antibodies is found in the antisheep hemolysins formed by the rabbit which have held my interest for the past seven years. As far as is known they act as do other cytolysins including such antibodies as trypanolysins. Parenthetically, I should add that we left the trypanolysins and started work with hemolysins because hemoglobin released from the red cell by hemolysis can be accurately measured by modern photometric methods. By using these methods with a 50% endpoint, we possess one of the most delicate

and quantitative measures of the biological activity of antibodies so far devised.

Since the pioneer work of Forssman (1911) and Landsteiner and Prašek (1912), it has been known that immunization of rabbits with sheep red cells yields hemolysins with two specificities: one with Forssman and the other with isophile (i.e., sheep) specificity. The experimental studies of Dr. D. W. Talmage and his associates and our group indicate that within each specificity there are two hemolysins which have markedly different biological characteristics and activities. The difference in the half life of these four activities is shown in figure 2. The genesis of this

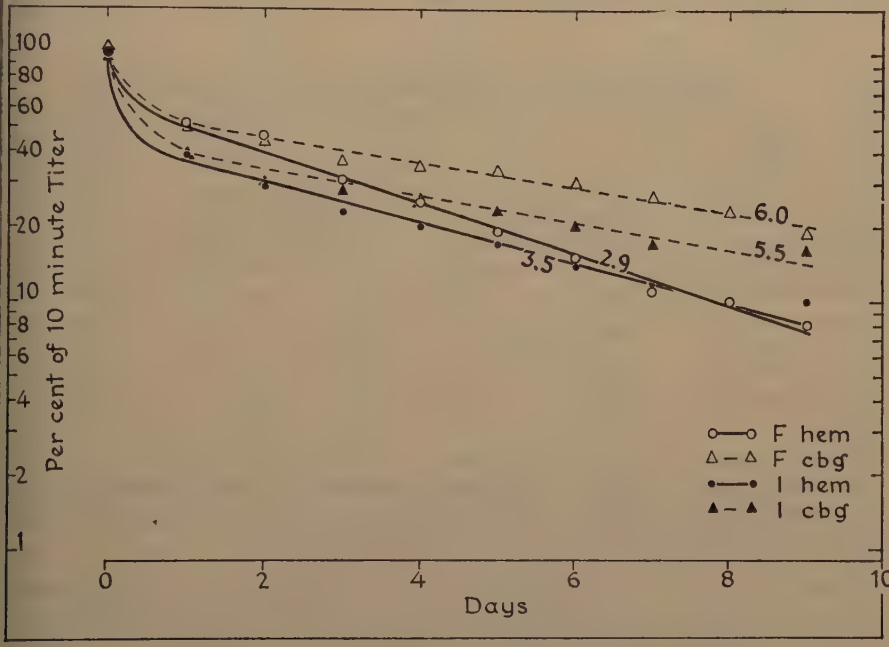


Fig. 2. The passive transfer into a normal rabbit of a rabbit antiserum containing four antibodies to sheep red cells. The anti-Forssman (F hem) and the isophile (I hem) hemolysins disappeared at faster rates (2.9 and 3.5 days) than the anti-Forssman (F cbg) and the isophile (I cbg) combining activities (6.0 and 5.5 days). From Taliaferro and Talmage, 1956.)

work was three-fold: the earlier work of Païc (1939) which indicated that the molecular weight of hemolysins was about 1,000,000 whereas that of most precipitins was about 160,000 (see Kabat and Mayer, 1948); my own interest in the biological half life of antibodies; and Dr. Talmage's interest in the relationship of the combining capacity and the hemolytic capacity of hemolysin in the presence of complement.

For simplicity I shall consider the two rabbit hemolysins of Forssman specificity as they are more easily studied because they predominate. One of these is a large molecule (ca 1,000,000 molecular weight) although small in total amount, is highly hemolytic, localizes during electrophoresis in the γ_1 (= T) or fast moving γ globulin fraction, and has a biological half life of 2.8 days. The other one is a smaller molecule (ca 165,000 molecular weight) although large in total amount, is slightly hemolytic, localizes during electrophoresis in the γ_2 or slow moving γ globulin and has a biological half life of 5.6 days. The foregoing characteristics are shown in table 1. During immunization, the large hemolytic antibody

TABLE 1

FORSSMAN ANTISHEEP RED CELL ANTIBODIES IN THE RABBIT*

Characteristics			Data from:*
Activity	Highly hemolytic	Weakly hemolytic	2
Molecular weight	1,000,000	165,000	1, 5
Rate of hemolysis	Varies as the square of the concentration	Varies as the 4th power of the concentration	6
Blood/tissue equilibration	$\frac{80}{20}$	$\frac{50}{50}$	3
Electrophoretic mobility	γ_1 (T fraction)	γ_2	1, 2
Peak titer	Early	Late	4
Half life	2.81 ± 0.12 days	5.56 ± 0.17 days	3

* The data were obtained from the following six references: (1) Stelos (1956); (2) Stelos and Talmage (1957); (3) Taliaferro and Talmage (1956); (4) Talmage et al. (1956a); (5) Talmage et al. (1956b); (6) Weinrach and Talmage (1958).

is formed first and reaches a high titer by the 5th day. The smaller slightly hemolytic one appears later and reaches a peak on about the 25th day at a time when the titer of the large one is decreasing. These differences are shown in figure 3. Although hemolytic units (measured by the amount of hemoglobin released) and combining units (measured by the ability of an antiserum to block the uptake of a standardized I^{131}-labeled antibody on red cells) actually include both antibodies, the tests can be used to differentiate the two because of their markedly different characteristics. Thus, the small antibody is so poorly hemolytic that it adds very little to the total amount of hemolysis. Conversely, the large antibody is proportionately so small in amount that it adds very little to the total combining capacity. Therefore, hemolysin titer can be ascribed to the large molecule and combining activity to the small one. The hemolytic efficiency is the hemolytic titer divided by the combining titer. As shown in figure 3, this efficiency declines as the combining titer

increases. The two types of hemolysin have also been found by Stelos (1958) with a bovine isophile specificity after immunizing rabbits with bovine cells.

The work of Weinrach and Talmage (1958) gives a probable explanation of the difference in hemolytic efficiency of the two types of antired-

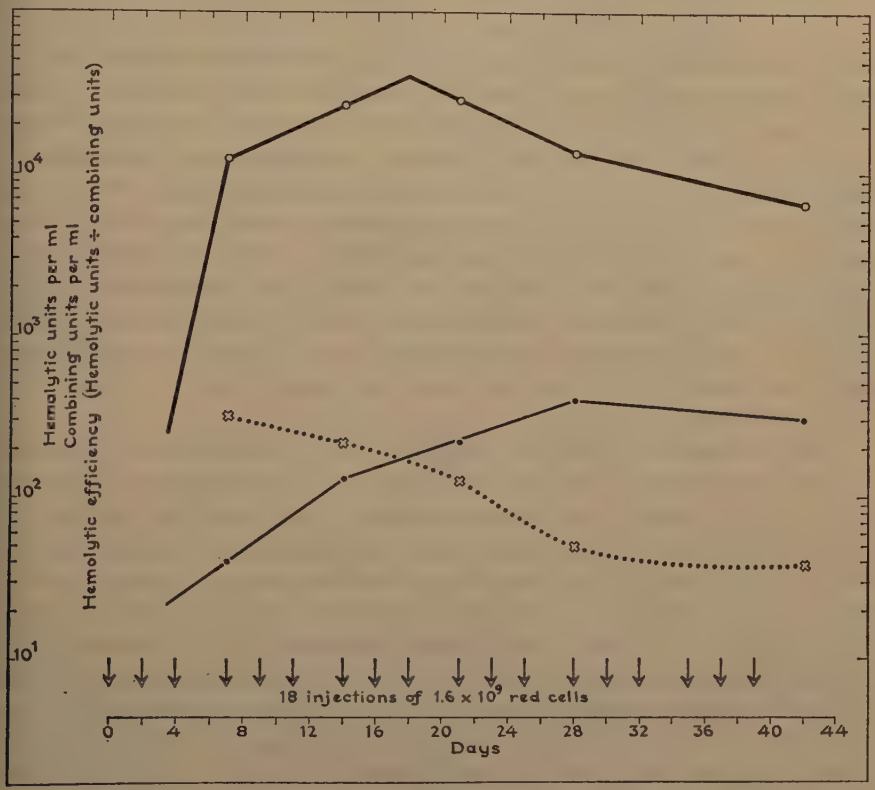


FIG. 3. The mean course of hemolysin (heavy solid line) and combining activity (fine solid line) together with the resultant hemolytic efficiency (dotted line) in the antiserums of 6 rabbits repeatedly injected intravenously with sheep red cells. These activities were of the anti-Forssman type. (Modified from Talmage et al, 1956a.)

cell antibody. For example, the rate of hemolysis varies with the square of the concentration of the larger molecule and as the fourth power of the smaller one. The results indicate that a two-molecule complex is necessary to produce a single hemolytic injury by the large antibody, whereas a four molecule complex is necessary to produce such an injury by the small one. There is, however, an anomaly in this comparison of hemolytic capacity. The comparison holds when titers are determined by the highest dilution that will give a specific effect because

there are too few molecules of either antibody to saturate the red cell. Under biological conditions, however, when a red cell is injected or a parasite enters the blood, there are frequently sufficient molecules of each antibody in the undiluted plasma to saturate the antigen and cause lysis almost instantaneously.

Two questions immediately arise. Are these two antibodies of identical or only of closely related immunological specificity? If they are of identical specificity, is the formation of two antibodies with such different biological activities characteristic of certain antigens (such as red cells) or of all antigens in the rabbit? Unfortunately, we can answer neither of these questions categorically. The two antibodies must be similar, if not identical, in specificity, because they cross-block each other, i.e., "saturation" of a red cell with one type prevents adsorption of the other, and it is possible for an avid small antibody to combine with so many closely adjacent sites on a cell that the action of the hemolytic antibody will be greatly inhibited. Whether every antigen injected into rabbits produces antibodies of the two molecular sizes cannot be answered so far, as we have at present no test for any antibody of the large molecular type except as a hemolysin.

The difference in molecular weight of the two hemolysins makes it possible to concentrate the hemolytic activity by centrifugation. Talmage et al (1956b) found a 4-fold concentration of the hemolytic activity as contrasted to a 2-fold concentration of combining capacity in the bottom third of a serum which was centrifuged for 3 hours in an average gravitational field of 105,000 $\times$ g. Stelos and Talmage (1957) used starch block electrophoresis to make a much sharper separation of the two types. As is clearly shown in figure 4, most of the hemolytic activity is in the γ_1 or T fraction, whereas most of the combining capacity is in the γ_2 fraction. This difference is strikingly shown by the hemolytic efficiency, i.e., hemolysin/combining ratio (= H/C) which is 834 in the γ_1 and only 9 in the γ_2 fraction.

The mechanism of hemolysis is a fascinating and complex process. It is generally assumed that the hemolytic antibody combines with the red cell and in the presence of complement induces the lytic injury. The work of Mayer et al (1948) indicates that, during this process, complement is destroyed whereas antibody dissociates and can initiate a series of lytic injuries on the same or other red cells provided sufficient complement is present. The degree of avidity of hemolysin as expressed in the rate of dissociation or movement from site to site on the same or different red cells is of great importance in lysis. Weinrach and Talmage (1958) suggest that there is an optimal avidity which gives maximum hemolysis. Below the optimum, the antibody would be relatively inefficient because it would frequently dissociate before lytic injury would be completed

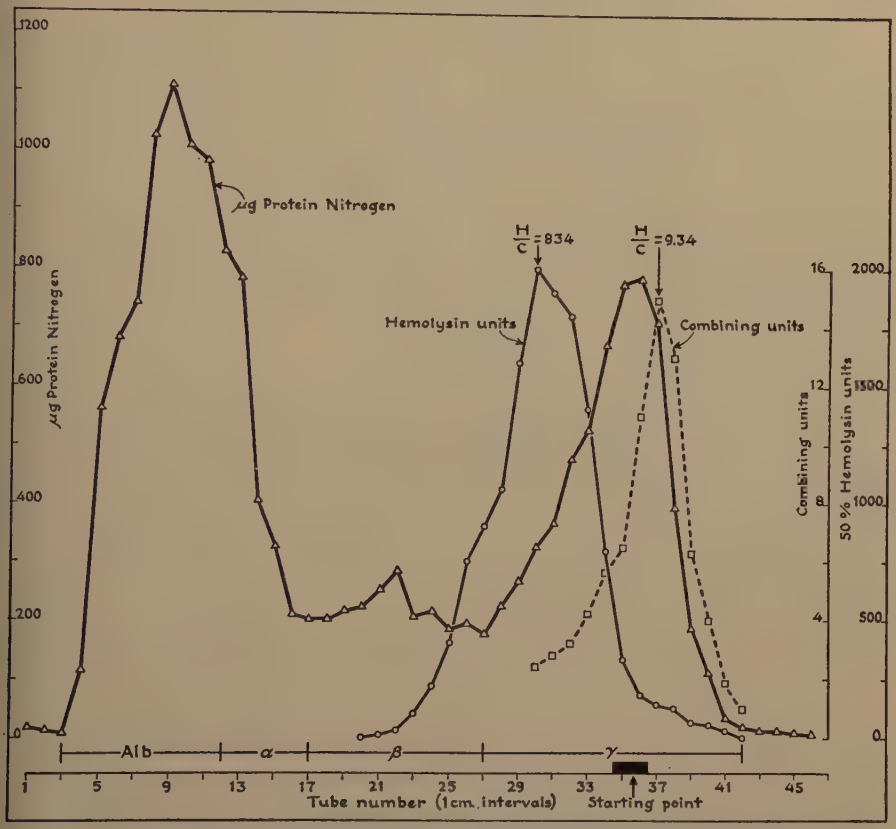


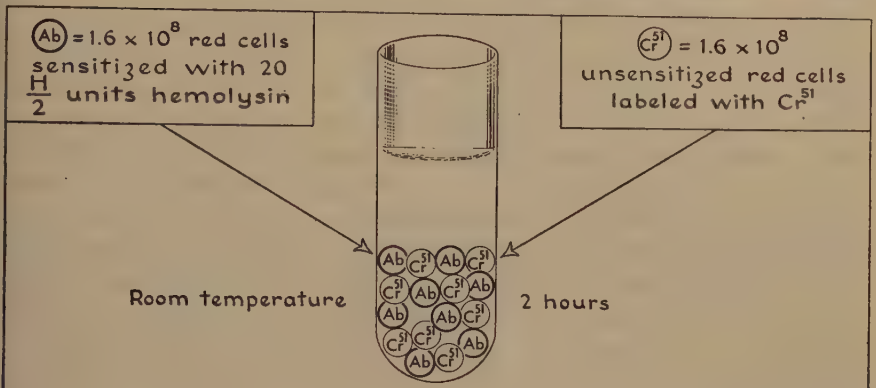
FIG. 4. The electrophoretic distribution of protein nitrogen and of Forssman hemolysin and combining activity in an antiserum from a rabbit 2 days after the last of 8 intravenous injections of 1.6×10^9 sheep red cells per kg during a 2-week period. (From Stelos and Talmage, 1957.) Peaks in hemolytic titer and combining titer were reached in such different fractions that the ratio of the two titers, i.e., the hemolytic efficiency (H/C), at the two peaks differed by a factor of over 90.

and, above the optimum, it would dissociate so slowly that it would produce only a few lytic injuries.

Avidity was first used to explain the difference in capacity of the diphtheria antitoxin to precipitate with toxin in vitro and to neutralize toxin in vivo (cf. Barr, 1951). It has been related to the rate of the secondary reactions, such as neutralization, precipitation and agglutination, and also to the equilibrium between antigen and antibody in the primary reaction. This equilibrium is probably modified by factors ranging from immunological valence, goodness of fit of the combining groups and solubility of the antigen-antibody complex. The avidity of antibody increases with time after a single injection of antigen and is especially high during an anamnestic response.

Bowman et al (1951) first demonstrated the transfer of hemolysin from both stromata and red cells to other red cells. It is generally assumed that intersite transfer on the same cell is more important than intercellular transfer but the latter probably gives a relative measure of the former. We (Taliaferro et al, 1958) have, therefore, developed a quantitative measure of intercellular transfer based on the work of Weinrach et al (1958). Rachromate ($Na_2\ Cr^{51}O_4$) was used to label red cells because Cr^{51} specifically labels hemoglobin and thus makes it possible to determine the hemoglobin derived from chromium-labeled cells. The actual measurement of intercellular hemolysin transfer was carried out as follows: Washed normal sheep red cells (1.6×10^8) are sensitized with twenty 50% units of a rabbit Forssman hemolysin. The sensitized unlabeled cells are put in contact with an equal number of unsensitized Cr^{51}-labeled cells for two hours at room temperature. During this interval, intercellular transfer of antibody can take place from sensitized unlabeled to unsensitized Cr^{51}-labeled cells. The mixture is then incubated at 37°C for 30 minutes with excess complement, and hemolysis is stopped abruptly with citrate. In the supernatant, total hemoglobin is determined photometrically and Cr^{51}-labeled hemoglobin is determined by gamma counts. The procedure and three possible results are given in figure 5. As shown in the first line of results, no antibody was transferred because 50% of the total cells were hemolysed, and no Cr^{51} occurred in the hemoglobin of the supernatant. Since the sensitized cells were not saturated with antibody, hemolysin which left them could attach itself to sensitized or to Cr^{51}-labeled cells at random. Thus, in the second line of possible results in figure 5, one 50% hemolytic unit was transferred because 75% of the total cells were hemolysed and 50% of the total Cr^{51} available was in the hemoglobin of the supernatant. In a study of various serums, we have found that 5 or more 50% hemolytic units transfer in nonavid serums from normal rabbits (which contain low-titered natural antibody) or from rabbits within 4 days after a single injection of heated sheep red cell stromata, whereas frequently less than one half of a hemolytic unit transfers in avid serums at peak titer during initial and anamnestic responses and for several weeks thereafter. The great increase in avidity which takes place shortly after 4 days following a single injection of antigen must chiefly involve the large hemolytic antibody since the small antibody appears later as shown in figure 3. It is thus evident that smaller amounts of nonavid serums are needed to produce a given amount of hemolysis under the conditions of our titration than are required with avid serums.

My coworker, Mr. Philip D'Alesandro (unpublished work) has found that both the ablastin which inhibits reproduction early in infections with


Ab = 1.6 x 10^8 red cells sensitized with 20 H/2 units hemolysin
Cr51 = 1.6 x 10^8 unsensitized red cells labeled with Cr51
Room temperature
2 hours

Complement added and hemoglobin and radioactivity of supernatant determined at 2, 4, 8, 15, and 30 minutes

Expected results at 30 minutes	Percent in supernatant	
	Hemoglobin	Cr^{51} counts
No antibody transfer	50	0
Transfer of 1 $\frac{H}{2}$ unit	75	50
Transfer of 4 $\frac{H}{2}$ units	90	80

FIG. 5. Schematic representation of the intercellular transfer of hemolysin (in terms of 50% units) from unlabeled sensitized to Cr^{51}-labeled unsensitized sheep red cells during 2 hours in vitro contact at 22°C. (From unpublished data by W. H. and L. G. Taliaferro and Pizzi.)

Trypanosoma lewisi and the lysin which terminates the infection (Taliaferro, 1924, 1932 and 1948) have about the same electrophoretic mobility and localize in the γ_1 or T fraction. The lytic activity is associated with a large molecule ($S_{20} = 16S$) similar to the hemolysin of high molecular weight, whereas preliminary work indicates that ablastic activity is associated with a small molecule ($S_{20} = 6S$) similar to the small hemolysin.

Recently, Terry (1957) found an innate principle in the serum of cotton rats which agglutinates and lyses *T. vivax* in vitro and appears to be responsible for the complete resistance of this species to infection with the trypanosome. Like the acquired lysin to *T. lewisi*, this innate lysin to *T. vivax* is found in the β and fast moving γ globulins (probably what we term the γ_1 or T fraction).

The preceding few examples show the diversity of antibodies in physico-chemical characteristics, their avidity in the first stage antigen-antibody union and their ability to induce and support the various secondary reactions. They also show that we have no method of measuring *total* antibody chiefly because we have no inclusive definition of antibody. In spite of this drawback, we have excellent *relative* methods of measuring some antibodies, i.e., the quantitative precipitin methods for avid precipitating antibodies, the antigen-binding capacity test of Farr (1956) for precipitating and nonprecipitating antibodies, the combining capacity test of Talmage and Freter (1956) for antired-cell antibodies and the 50% end point test (cf. W. H. and L. G. Taliaferro, 1950 and 1956) for hemolysins.

The lack of methods for measuring total antibody has not hindered the accumulation of considerable data on the synthesis of the antibody molecule. Most of these data parallel the data on the synthesis of other mammalian proteins and the induced enzyme proteins of bacteria.

There seems little doubt that all or part of the antigen molecule induces the body to start or to increase the production of antibody. Several theories have been advanced as to how this induction can take place (see Burnet and Fenner, 1949, Burnet, 1956 and 1958, Jerne, 1955 and 1956, and Talmage, 1957b), but experimental evidence is lacking. There is no agreement as to whether antibody is the increased production of a normal globulin which happens to react with the antigen, or a slightly modified normal globulin, or an entirely new globulin.

Recently, Jaroslow and I have probably found some of the materials which must be present to initiate antibody formation. We assumed from X-ray studies that irradiation destroys something necessary for the initiation (possibly the induction) of the immune process. Thus, it had been known for some time that early stages of the immune process are X-ray sensitive, whereas later stages are relatively X-ray resistant (review by W. H. and L. G. Taliaferro, 1951a, and Kohn, 1951). Dixon et al (1952a) and Taliaferro et al (1952) showed that the early radiosensitive period could only be a few hours in duration and might be much shorter. Later, we (W. H. and L. G. Taliaferro, 1954) found that the power to form hemolysin decreases with an apparent half life of about 2 hours as tested in groups of rabbits injected with 2×10^7 sheep red cells before and after total body administration of 500 r. The data are shown in figure 6. In 1955 and 1956, it had been further shown by the Harrises and their associates that antibody formation can be restored in X-rayed (425 r) rabbits when the antigen is injected with lymph node cells either separately or after incubation together. In a somewhat similar manner, Jaroslow and I (1956) found that the hemolysin-producing capacity is re-

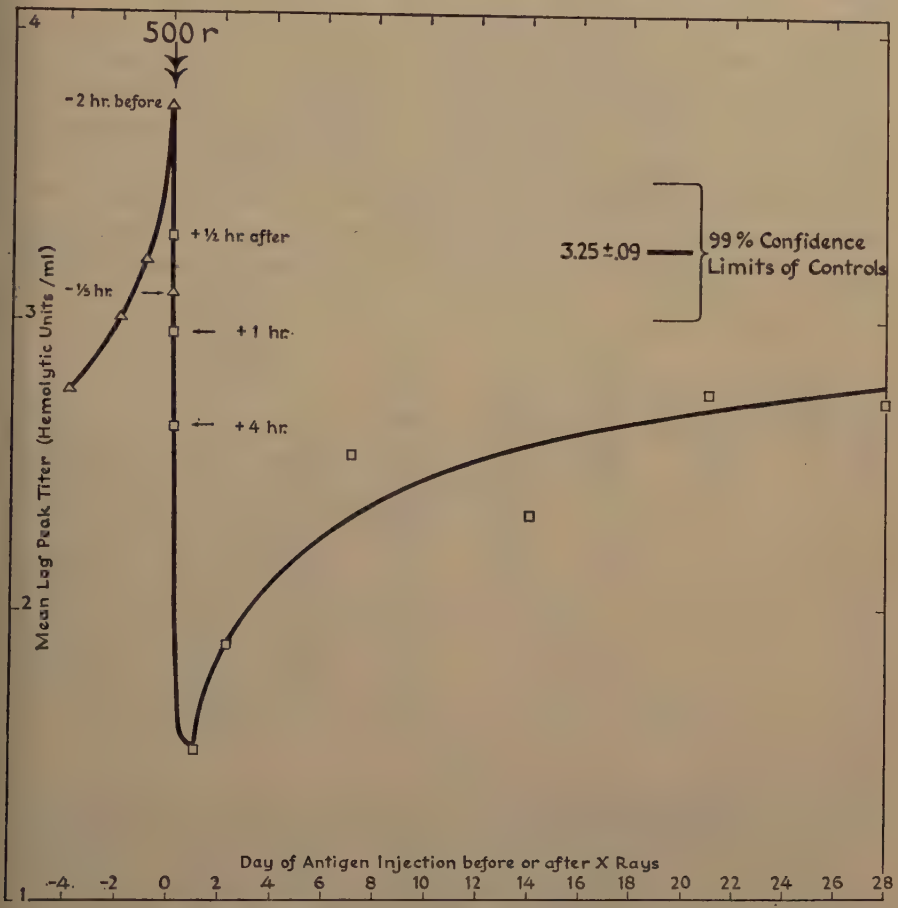


FIG. 6. Mean log peak hemolysin titer in the antiserums from 14 groups of rabbits as related to the time of the intravenous injection of 2×10^7 sheep red cells per kg rabbit 4 days before through 28 days after total body irradiation. (Redrawn from W. H. and L. G. Taliaferro, 1954.) Note the precipitous drop in peak titer when antigen was given from 2 hours before to 4 hours after irradiation.

stored to a small but significant extent when sheep red cells are mixed and injected with a mince of normal rabbit spleen into rabbits irradiated one day previously with 400 r. We soon found, however, that the restoration is not mediated by specific cells and does not require intact cells. Mince of mouse spleen is as effective as rabbit spleen, and HeLa cells are even more effective. Furthermore, certain tissue and cell extracts are satisfactory. Then yeast autolysate was found to give essentially complete restoration. Minces of rabbit kidney or of rabbit muscle give essentially negative results. The experimental data from treated groups

of X-rayed rabbits with controls are shown in figure 7. Unpublished work indicates that restoration of the hemolysin-forming capacity can be effected in irradiated rabbits to the same degree as splenic mince by products of the action of the specific nuclease on ribonucleic acid or on deoxyribonucleic acid. Neither nucleic acid has been found active. The only chemically defined nucleic acid derivative we have found to equal

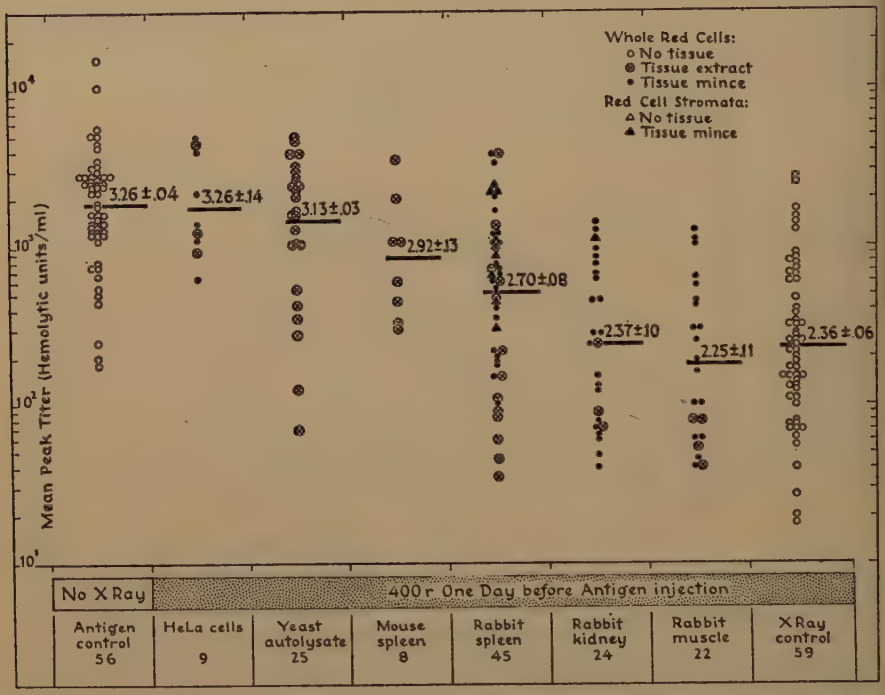


FIG. 7. Individual and mean log peak hemolysin titers in the antiserums from 6 groups of rabbits receiving total body irradiation before the intravenous injection of sheep red cells or heated stromata and various additional substances. Data are also included from nonirradiated and irradiated control groups receiving antigen alone. (Modified from Jaroslow and Taliaferro, 1956, with additional data.)

the activity of splenic mince is kinetin (= 6-aminofurfurylpurine), a derivative of deoxyribonucleic acid. We have no explanation of how the restorative materials act, but it seems likely that the yeast autolysate contains kinetin or other nucleic acid derivatives together wih a further substance which mediates some interaction between antigen and nucleic acid derivative. The whole process is probably a part of the nucleic acid control of protein synthesis.

Some of our recent experiments also indicate that these restorative substances when injected with sheep red cells into nonirradiated rabbits

lead to above normal amounts of hemolysin. At present, we interpret these results to indicate that necessary compounds are in marginal amounts at critical sites after irradiation and are below an optimal concentration in normal animals.

There is a difference of opinion among immunologists as to whether or not antibody formation occurs in the complete absence of antigenic stimulation. Our own work on hemolysins indicates that it cannot. Thus, figure 8 shows the daily output of hemolysin, corrected for metabolic

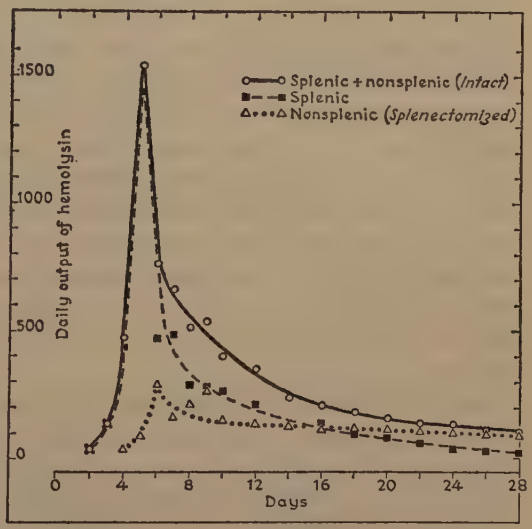


FIG. 8. Daily output of splenic and nonsplenic hemolysin calculated from hemolysin curves in normal and in splenectomized rabbits after a single intravenous injection of 1.6×10^9 sheep red cells per kg. (From Taliaferro, 1955.) Note that production by the spleen reached a high peak rapidly and then decreased rapidly while that by nonsplenic sources rose later to a lower peak and decreased slowly.

decay, by the spleen and by nonsplenic sources in the rabbit following a single intravenous injection of antigen. In this figure, nonsplenic output is considered to be that of splenectomized animals, and splenic output is assumed to be the difference between the daily output in intact and in splenectomized animals. The daily output of splenic hemolysin rises rapidly and falls off sharply (half life of about 2 days). The sharp decline in production is connected with the absence of antigenic stimulation since a second injection of antigen produces a second cycle of splenic activity comparable to the first (W. H. and L. G. Taliaferro, 1952). Nonsplenic hemolysin reaches a lower peak later and decreases more slowly. Actually, antibody production by nonsplenic sources for 3 weeks about equals that of the spleen for 1 week. The correlation between rate and length of antibody synthesis suggests that antigen is exhausted during the process of antibody formation.

If the antigen as a whole or in part is the actual inducer, the situation may be somewhat analogous to that governing the inducible enzymes of

bacteria. A few molecules of these enzymes are formed by the bacteria without the inducer, but a greatly accelerated production follows in the presence of the inducer. Furthermore, except in special cases, the inducer must be present for continued accelerated production.

During a long-continued regimen of antigenic stimulation, antibody decreases. This decrease may in part be associated with the absorption of antibody by antigen. In some systems, there may be a paralysis of the immune mechanism, as described by Felton (1949). Conversely, antigen may remain in the body but for various reasons may not effectively stimulate antibody formation. In our own work (1951b and 1952) involving injections of red cells into rabbits for two or three months, the spleen forms more hemolysin than after a single injection, but its output falls off in much the same way as after a single injection. Further experiments indicate that the decline in serum antibody is not caused by absorption or by a paralysis of the immune mechanism. It is a true refractory stage which occurs during antigenic stimulation and is in part controlled by the avidity and amount of circulating antibody. Thus, Talmage, Freter and Thomson (1956) found that the hemolysin response is greatly reduced when a mixture of red cells and serum from a refractory rabbit is injected into nonimmunized rabbits (see also Barr et al, 1950, and Mason et al, 1955).

Serum antibodies as well as other serum proteins, once formed, undergo metabolic degradation into their constituent amino acids which are thus added to the amino acid pool and become available for the synthesis of other proteins. This process is frequently termed recycling. In general, the rate of metabolic decay is faster in small animals such as the mouse with high metabolic rates than in larger animals such as the rabbit with low metabolic rates. Metabolic rate is only one factor, however, because antibodies decay more slowly in children with their higher metabolic rate than in adults (Dixon et al, 1952b). Even more striking is the fact already mentioned that two antibodies with closely related, if not identical, immunological specificity, may be formed in the same animal and decay at different rates (table 1 and fig. 2). In spite of the many factors influencing the rate of decay, the rate for a given antibody in a given species is remarkably constant under laboratory conditions as is illustrated by the small standard errors shown in table 1. Previous immunization and, hence, the presence of the synthesizing mechanism does not modify the rate of metabolic decay (Humphrey and McFarlane, 1954, and Taliaferro and Talmage, 1956). Moreover, antibodies and other serum proteins have remarkably fast metabolic turnovers in mammals (cf. Whipple 1956). At the other extreme, collagen has an extremely slow turnover (Neuberger et al, 1951, and Thompson and Ballou, 1956).

Although antibody is constantly being degraded into its constituent amino acids, there is no exchange between its amino acids and certain of its atoms and homologous amino acids and atoms in other compounds of the body *as long as the antibody retains its immunological reactivity.* This conclusion was first reached by Heidelberger and his associates (1942). It is best shown by passively transferring isotopically labeled antibody to an unlabeled recipient (see Gros et al, 1952b, Dubert et al, 1953, Humphrey and McFarlane, 1954, and the review by Taliaferro, 1957). Figure 9 illustrates an experiment of this type with S^{35}-labeled antiserum (Taliaferro and Talmage, 1955). The specific radioactivity of antibody in the labeled donor was 40.6 counts per minute per μg. antibody nitrogen. When introduced into the recipient, which was unimmunized and therefore neither contained nor made unlabeled antibody to dilute the radioactivity, the S^{35}-labeled antibody retained its original radioactivity for 9 days. During this time, the antibody was constantly decaying and only about 25% of the amount originally injected was pres-

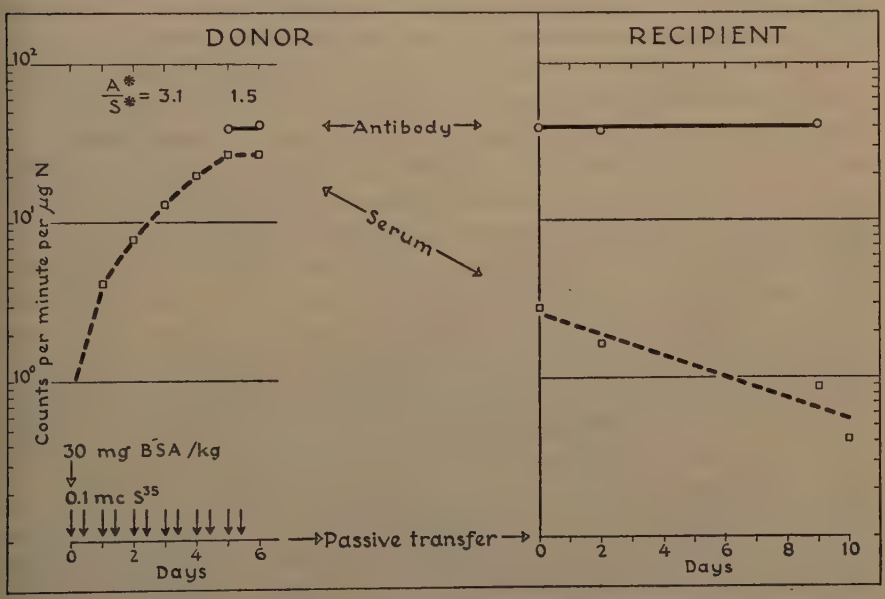


Fig. 9. The passive transfer of an antiserum containing S^{35}-labeled antibovine-serum-albumin and S^{35}-labeled serum proteins to a normal recipient. The S^{35} counts per μg antibody N (solid lines) remained constant in the donor and recipient whenever antibody determinations could be made while the S^{35} counts per μg serum protein N (dash lines) increased in the donor ($A^*/S^* = 3.1$ on day 3 and 1.5 on day 5 and 6) and decreased in the recipient. Precipitin titer is not shown in the figure. It rose rapidly beginning on day 3 in the donor and declined in the recipient. (From Taliaferro and Talmage, 1955.)

ent on the ninth day. It is obvious, therefore, that no atomic sulfur or nonlabeled sulfur-containing amino acids replaced a significant amount of the istopically labeled sulfur in the antibody. The stability of the antibody molecule is important because upon it are based some of the methods used in the study of antibody synthesis. The recipient, on the other hand, contains and makes unlabeled nonantibody serum proteins which immediately and progressively dilute the S^{35}-labeled nonantibody serum proteins as shown in figure 9.

Irrespective of whether antibody formation is the result of the increased formation of selected normal globulins or the synthesis of a new globulin, it is synthesized de novo in that the antibody molecule is formed directly from the amino acids of the amino acid pool (Green and Anker, 1954, and review by Taliaferro, 1957). It is not formed directly from preexisting globulins or other proteins (Gros et al, 1952a, Dubert et al, 1953, and Askonas et al, 1956). In this respect, it is similar to the enzyme proteins of bacteria (Rotman and Spiegelman, 1954, Hogness et al, 1955, and Monod, 1956). The actual time of synthesis is probably very short. Long-lived amino acid-containing precursors during synthesis were postulated earlier for antibodies and other proteins, but probably do not occur.

Long-lived precursors of antibodies would seem most likely to occur during the induction period of the immune process, i.e., after the introduction of the antigen but before the appearance of serum antibody. In fact, they seemed to account for the findings of Green and Anker in 1954. These authors administered three different isotopically labeled amino acids during the secondary response to ovalbumin—two during the induction period and one during the rise of serum antibody. The relative concentration of isotopes in antibody removed on the 5th day led these authors to conclude that 31% of the amino acids in the antibody were withdrawn from the amino acid pool during the induction period and the remainder from the pool during the rise of antibody in the serum.

Different results were obtained by Taliaferro and Talmage (1955), Stravitsky (1956 and 1957) and W. H. and L. G. Taliaferro (1957). As shown in figure 10, we divided the secondary immune response to bovine serum albumin between two rabbits. The induction period was passed in a donor which received one intravenous reinjection of BSA and twice daily doses of S^{35}-labeled amino acids. The spleen of the donor before precipitins appeared was then removed, minced and injected intravenously into an unimmunized unlabeled recipient. Antibody probably began to be made immediately in the recipient, but was labeled to a very small percent of that of the donor antibody. Parenthetically, it should be noted that we can be sure that antigen in the transferred spleen was

not responsible for the antibody made in the recipient because the antibody was all synthesized before 6 days which is the earliest time antibody appears after an initial injection of antigen (cf. Knox and Endicott, 1950).

The reciprocal relationship between the fall of specific radioactivity and the increase in antibody in the recipient (fig. 10) indicates that the recipient made small amounts of highly labeled antibody at first from the S^{35}-labeled materials carried over in the spleen from the donor and then made unlabeled antibody from the recipient's own amino

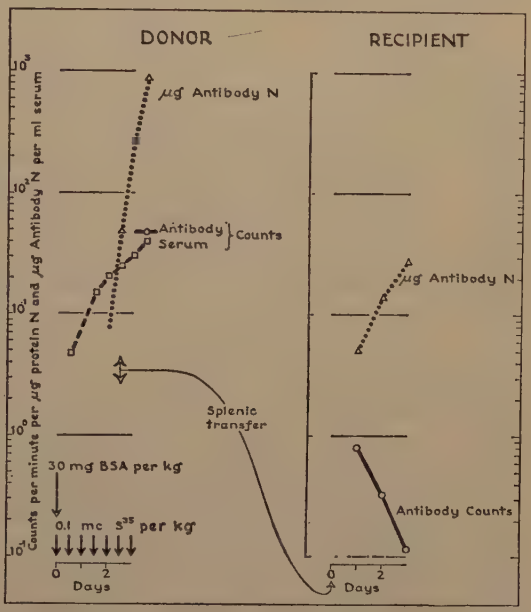


Fig. 10. The transfer to a normal recipient of spleen mince from a donor, 2.5 days after an intravenous injection of bovine serum albumin (= BSA) and after 5 intravenous injections of S^{35}-labeled amino acids. (Data from W. H. and L. G. Taliaferro, 1957.) In the recipient, precipitins (dotted lines) increased from day 1 (first determination) through day 3 while the S^{35} counts per μg antibody N (solid lines) were markedly lower than those in the donor on day 1 and decreased through day 3. The S^{35} counts per μg serum protein N (dash line) increased in the donor as in figure 9.

acids which progressively diluted the S^{35}-labeled antibody. On the third day, the S^{35}-labeling of the recipient antibody was only 0.02% of the specific radioactivity of the donor antibody. Thus, not more than 0.02% of the amino acids of the recipient antibody could have been drawn from the amino acid pool of the donor. We (Taliaferro and Talmage, 1955) accounted for Green and Anker's results by assuming that isotopic-labeled complement and coprecipitating antibody were carried down and included with the precipitating antibody removed on the 5th day. Dixon et al (1956) suggested that their results may have been due to recycling. Both factors were probably operative.

The transit time is the period elapsing between the administration of isotopically labeled amino acids and their presence in antibody in sufficient concentration to be demonstrated by laboratory procedures. During

the rapid rise of the secondary response to bovine serum albumin in the rabbit, Mrs. Taliaferro and I (1957) have found this interval to be not less than 20 minutes and not more than 40 minutes. Figure 11 shows the beginning and subsequent rapid rise in the incorporation of S^{35} amino acids into antibody. It seems significant that our determination of the

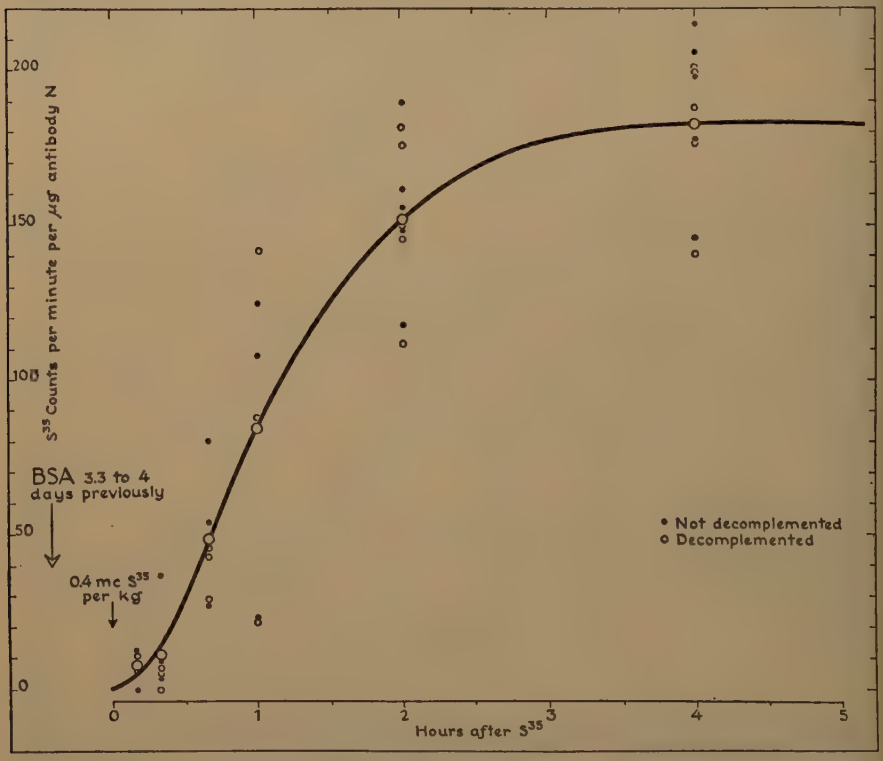


FIG. 11. The incorporation of S^{35}-labeled amino acids into antibovine-serum-albumin (= antiBSA) in rabbits during a rapid secondary rise of precipitins as shown by S^{35} counts of antibody for 5 hours after one intravenous injection of S^{35}-labeled amino acids. The large circles are mean values of the smaller circles and dots which were obtained with and without decomplementing the antiserums before testing. (From W. H. and L. G. Taliaferro, 1957.)

transit time in vivo is almost identical with that obtained for the incorporation of C^{14} in vitro by Askonas and White (1956). Thus, there seem to be no important factors holding up antibody in vivo that do not act in vitro. This length of time also agrees excellently with the approximate 30-minute transit time reported by Peters (1953) for the formation in vitro of chicken albumin, by Green and Anker (1955) for the formation of serum proteins in vivo in the rabbit, by Junqueira et al (1955) for the

formation of pancreatic proteins in vivo in the rat and by Askonas et al (1956) for the formation of γ globulin in vivo in the rabbit.

The period of not more than 40 minutes represents the maximum time necessary to assemble amino acids into rabbit antibody. It thus includes the maximal length of life of intermediates as well as the time necessary for amino acids to penetrate the antibody-forming cells and for antibody to leave the cells and reach the serum. The actual time of synthesis and, therefore, the maximum length of life of intermediates must be less than 40 minutes and may be only a few minutes as indicated for the induced bacterial enzymes (Hogness et al, 1955).

The absence of long-lived amino acid precursors and the short transit time indicate that, for antigens injected intravenously into the rabbit, serum titers suitably corrected for metabolic decay give a good approximation of the rate of antibody synthesis. Indirect evidence that this is true is given by the similarity of the results of Dixon et al (1956) on the rate of synthesis of antibovine serum albumin in the rabbit as measured by isotopic methods and our results with hemolysins using the changes in serum titer corrected for metabolic decay. Dixon and his associates reported that the rate of synthesis after peak titer fell off rapidly (half life of 1.3 days) during first and second responses. These data are obviously similar to our results during the primary hemolysin response for both intact and splenectomized rabbits, as shown in figure 8 (see also Taliaferro, 1955 and 1956). Limited direct evidence is also shown by our finding (W. H. and L. G. Taliaferro, 1957) that a single dose of S^{35}-labeled amino acids given when different amounts of antibody are being formed results in approximately the same specific radioactivity of the antibody (fig. 12). Such a similarity would not occur if stored unlabeled antibody was being released unless it was released at a constant rate over the period studied.

When antibody formation occurs in granulomata as after the use of Freund's adjuvant, antibody may be stored. Hence, the above relationships would not hold. Even after the intravenous injection of antigen, however, Cheng and Haurowitz (1957) believe that antibodies are formed in large amounts during a primary response and are later released after the initiation of the secondary response.

In summary, the induction of antibody formation (= preinduction period) at the beginning of the immunological induction period may involve some reaction between antigen and nucleotides although little is known about the process. Most of the so-called immunological induction period is concerned with the development of the antibody-forming mechanism. Once operative, this mechanism synthesizes antibody directly from the amino acids of the amino acid pool without the intervention of

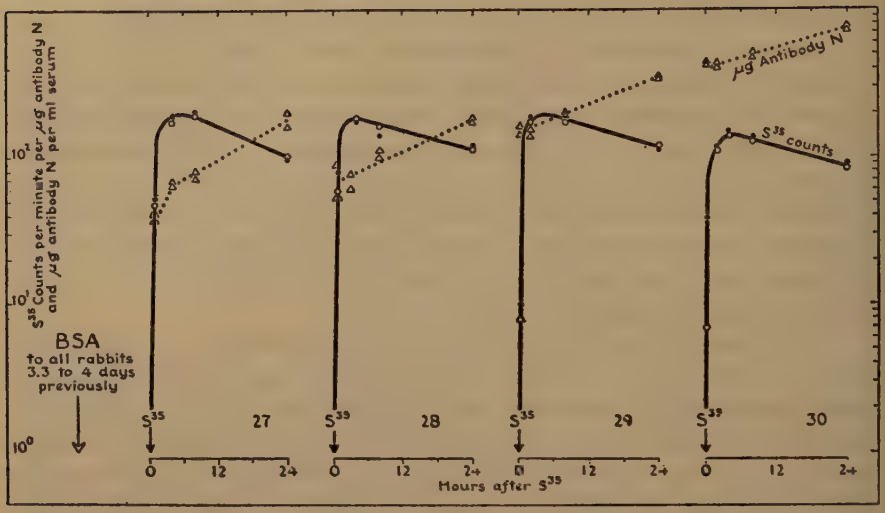


FIG. 12. Data from 4 of the rabbits in figure 11 showing the rise and fall in uptake of S^{35}-labeled amino acids by antibody during the 24-hour interval after one injection of S^{35}-labeled amino acids while precipitins were rapidly increasing in amount. (From W. H. and L. G. Taliaferro, 1957.) Note that the rise and fall in specific radioactivity (solid lines) were more or less uniform although the 4 rabbits were forming different amounts of antibody (dotted lines).

long-lived amino acid precursors. Antibodies are not formed by modifying preexisting proteins. The time of synthesis is less than 40 minutes and may be much less. This short length of time, as ascertained by the intravenous injection of isotopically labeled amino acids, indicates that serum titers under such conditions, suitably corrected for metabolic decay, give a good approximation of the actual rate of synthesis. Antibodies of the same immunological specificity made in the same animal may differ widely in their physico-chemical characteristics, their rate of metabolic decay, their avidity, their primary union with antigen and their ability to trigger and support the secondary immunological reactions. Such diversity makes it difficult to characterize antibody and impossible to measure total antibody in the serum although excellent relative measures are available.

REFERENCES

Ackroyd, J. F. 1949. The pathogenesis of thrombocytopenic purpura due to hypersensitivity to sedormid (allyl-isopropyl-acetyl-carbamide). *Clin. Sci.* 7, 249-283.

Askonas, B. A., Humphrey, J. H., and Porter, R. R. 1956. On the origin of the multiple forms of rabbit γ-globulin. *Biochem. J.* 63, 412-419.

Askonas, B. A., and White, R. G. 1956. Sites of antibody production in the guinea-pig. The relation between *in vitro* synthesis of anti-ovalbumin and γ-globulin and distribution of antibody-containing plasma cells. *Brit. J. Exp. Pathol.* 37, 61-74.

Barr, M. 1951. The formation of abnormal diphtheria antitoxin in the early stages of immunization. *J. Pathol. & Bacteriol.* **63**, 557-570.

Barr, M., Glenny, A. T., and Randall, K. S. 1950. Diphtheria immunization in young babies. *Lancet* **1**, 6-10.

Blacklock, D. B., Gordon, R. M., and Fine, J. 1930. Metazoan immunity: a report on recent investigations. *Ann. Trop. Med. & Hyg.* **24**, 5-54.

Bowman, W. M., Mayer, M. M., and Rapp, H. J. 1951. Kinetic studies on immune hemolysis. II. The reversibility of red cell-antibody combination and the resultant transfer of antibody from cell to cell during hemolysis. *J. Exp. Med.* **94**, 87-110.

Burnet, F. M. 1956. *Enzyme, Antigen and Virus.* Cambridge University Press, Cambridge, England.

Burnet, F. M. 1958. The modification of Jerne's theory of antibody production using the concept of clonal selection. *Australian J. Sci.* In press.

Burnet, F. M., and Fenner, F. 1949. *The Production of Antibodies.* 142 pp. Macmillan and Co. Ltd., London.

Cheng, H. F., and Haurowitz, F. 1957. Incorporation of S^{35}-amino acids into antibodies in primary and secondary response. *Fed. Proc.* **16**, 409-410.

Coventry, F. A., and Taliaferro, W. H. 1928. Hypersensitiveness to helminth proteins. I. Cutaneous tests with proteins of ascaris, hookworm and trichuris in Honduras. *J. Prev. Med.* **2**, 273-288.

Dixon, F. J., Maurer, P. H., Weigle, W. O., and Deichmiller, M. P. 1956. Rates of antibody synthesis during first, second and hyperimmune responses of rabbits to bovine gamma globulin. *J. Exp. Med.* **103**, 425-438.

Dixon, F. J., Talmage, D. W., and Maurer, P. H. 1952*a*. Radiosensitive and radioresistant phases in the antibody response. *J. Immunol.* **68**, 693-700.

Dixon, F. J., Talmage, D. W., Maurer, P. H., and Deichmiller, M. 1952*b*. The half-life of homologous gamma globulin (antibody) in several species. *J. Exp. Med.* **96**, 313-318.

Dubert, J. M., Gros, P., Coursaget, J., and Macheboeuf, M. 1953. Recherches diverses sur les anticorps effectuées grâce aux isotopes du carbone de masses atomiques 13 et 14. *J. Med. de Bordeaux* **130**, 303-309.

Farr, R. S. 1956. Measurement of antigen-combining capacity of antiserums as determined by ammonium sulfate precipitation of I^{131} antigen after complex formation with antibody. *Fed Proc.* **15**, 586.

Felton, L. D. 1949. The significance of antigen in animal tissues. *J. Immunol.* **61**, 107-117.

Forssman, J. 1911. Die Herstellung hochwertiger specifischer Schafhämolysine ohne Verwendung von Schafblut. Ein Beitrag zur Lehre von heterologer anti-körperbildung. *Biochem. Ztschr.* **37**, 78-115.

Green, H., and Anker, H. S. 1954. On the synthesis of antibody protein. *Biochim. et Biophys. Acta.* **13**, 365-373.

Green, H., and Anker, H. S. 1955. Kinetics of amino acid incorporation into serum proteins. *J. Gen. Physiol.* **38**, 283-293.

Gros, P., Coursaget, J., et Macheboeuf, M. 1952*a*. Recherches sur l'existence de précurseurs protéiques dans la formation des anticorps. Travail effectué avec de la valine marquée par le carbone 14. *Bull. Soc. Chim. Biol.* **34**, 1071-1073.

Gros, P., Coursaget, J., et Macheboeuf, M. 1952*b*. Differences dans le comportement métabolique des anticorps dans l'immunité active et dans l'immunité passive. Recherches effectuées au moyen d'isotopes indicateurs: carbone 13 et carbone 14. *Bull. Soc. Chim. Biol.* **34**, 1074-1077.

Harris, S., and Harris, T. N. 1955. Studies on the transfer of lymph node cells. V. Transfer of cells incubated *in vitro* with suspensions of *Shigella paradysenteriae*. *J. Immunol.* **74**, 318-328.

Harris, S., Harris, T. N., Ogburn, C. A., and Farber, M. B. 1956. Studies on the transfer of lymph node cells. VII. Transfer of cells incubated *in vitro* with filtrates

of trypsin-treated suspensions of *Shigella paradysenteriae*. *J. Exp. Med.* **104**, 645-661.

Harris, T. N., Harris, S., and Farber, M. B. 1955. Studies on the transfer of lymph node cells. VI. Transfer of cells incubated *in vitro* with *Shigella*-treated rabbit serum. *J. Immunol.* **75**, 112-122.

Harris, T. N., Harris, S., and Farber, M. B. 1956. Studies on the transfer of lymph node cells. VIII. The transfer of cells from blood and peritoneal exudates incubated *in vitro* with antigenic material derived from *Shigella paradysenteriae*. *J. Exp. Med.* **104**, 663-673.

Heidelberger, M., Treffers, H. P., Schoenheimer, R., Ratner, S., and Rittenberg, D. 1942. Behavior of antibody protein toward dietary nitrogen in active and passive immunity. *J. Biol. Chem.* **144**, 555-562.

Hogness, D. S., Cohn, M., and Monod, J. 1955. Studies on the induced synthesis of β-galactosidase in *Escherichia coli:* the kinetics and mechanism of sulfur incorporation. *Biochim. et Biophys. Acta.* **16**, 99-116.

Humphrey, J. H. and McFarlane, A. S. 1954. Rate of elimination of homologous globulins (including antibody) from the circulation. *Biochem. J.* **57**, 186-191.

Jaroslow, B. N. and Taliaferro, W. H. 1956. The restoration of hemolysin-forming capacity in X-irradiated rabbits by tissue and yeast preparations. *J. Infect. Dis.* **98**, 75-81.

Jerne, N. K. 1955. The natural-selection theory of antibody formation. *Proc. Nat. Acad. Sci.* **41**, 849-857.

Jerne, N. K. 1956. The development of the phage-inactivating properties of serum during the course of specific immunization of an animal: reversible and irreversible inactivation. *J. Immunol.* **76**, 200-208.

Junqueira, L. C. U., Hirsch, G. C. and Rothschild, H. A. 1955. Glycine uptake by the proteins of the rat pancreatic juice. *Biochem. J.* **61**, 275-278.

Kabat, E. A. and Mayer, M. 1948. *Experimental Immunochemistry*. Charles C Thomas, Springfield, Illinois.

Knox, W. C. and Endicott, F. C. 1950. I^{131} as an antigen label in the circulating serum of nonimmune rabbits. *J. Immunol.* **65**, 523-528.

Kohn, H. I. 1951. Effect of X-rays upon hemolysin production in the rat. *J. Immunol.* **66**, 525-533.

Kuhns, W. J. 1954. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. IV. Differences between human precipitating and non-precipitating skin-sensitizing diphtheria antitoxin as shown by electrophoresis. *J. Exp. Med.* **99**, 577-588.

Kuhns, W. J. 1955. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. VI. Further investigations on the identity and specificity of non-precipitating and skin-sensitizing antitoxin. *J. Exp. Med.* **101**, 109-117.

Kuhns, W. J. and Pappenheimer, A. M., Jr. 1952*a*. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. I. Relationship of skin sensitivity to purified diphtheria toxoid to the presence of circulating, non-precipitating antitoxin. *J. Exp. Med.* **95**, 363-374.

Kuhns, W. J. and Pappenheimer, A. M., Jr. 1952*b*. Immunochemical studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. II. A comparison between the immunological properties of precipitating and non-precipitating (skin-sensitizing) antitoxins. *J. Exp. Med.* **95**, 375-392.

Landsteiner, K. and Prašek, E. 1912. Ueber die bindenden und immunisierenden Substanzen der Blutkörperchen. *Ztschr. Immunitätsforsch.* **13**, *Orig.*, 403-420.

Mason, J. H., Robinson, M. and Christensen, P. A. 1955. The active immunization of guinea-pigs passively immunized with homologous antitoxic serum. *J. Hyg.* **53**, 172-179.

Mayer, M. M., Croft, C. C. and Gray, M. M. 1948. Kinetic studies on immune hemolysis. I. A method. *J. Exp. Med.* **88**, 427-444.

Monod, J. 1956. Remarks on the mechanism of enzyme induction. In *Enzymes: Units of Biological Structure and Function.* Ed. O. H. Gaebler: pp. 7-28. Academic Press, Inc. New York.

Neuberger, A., Perrone, J. C. and Slack, H. G. B. 1951. The relative metabolic inertia of tendon collagen in the rat. *Biochem. J.* **49**, 199-204.

Païc, M. 1939. Ultracentrifugation de l'hémolysine. Détermination de sa constante de sédimentation et de son poids moléculaire. *Bull. Soc. Chim. Biol.* **21**, 412-421.

Peters, Jr., T. 1953. Evidence of intermediate compounds in serum albumin synthesis. *J. Biol. Chem.* **200**, 461-470.

Rotman, B. and Spiegelman, S. 1954. On the origin of the carbon in the induced synthesis of β-galactosidase in *Escherichia coli. J. Bacteriol.* **68**, 419-429.

Sarles, M. P. 1938. The in vitro action of immune serum on the nematode, *Nippostrongylus muris. J. Infect. Dis.* **62**, 337-348.

Sarles, M. P. and Taliaferro, W. H. 1936. The local points of defense and the passive transfer of acquired immunity to *Nippostrongylus muris* in rats. *J. Infect. Dis.* **59**, 207-220.

Stavitsky, A. B. 1956. Mechanisms of antibody synthesis by transplanted cells. *Fed. Proc.* **15**, 615.

Stavitsky, A. B. 1957. Mechanism of the secondary antibody response to proteins. *Fed. Proc.* **16**, 652-660.

Stelos, P. 1956. Electrophoretic and ultracentrifugal studies of rabbit hemolysins. *J. Immunol.* **77**, 396-404.

Stelos, P. 1958. Comparative study of hemolysins to various antigens. I. Hemolysins to beef red cells. *J. Infect. Dis.* **102**, In press.

Stelos, P. and Talmage, D. W. 1957. The separation by starch electrophoresis of two antibodies to sheep red cells differing in hemolytic efficiency. *J. Infect. Dis.* **100**, 126-135.

Taliaferro, W. H. 1924. A reaction product in infections with *Trypanosoma lewisi* which inhibits the reproduction of the trypanosomes. *J. Exp. Med.* 39, 171-190.

Taliaferro, W. H. 1932. Trypanocidal and reproduction-inhibiting antibodies to *Trypanosoma lewisi* in rats and rabbits. *Amer. Jour. Hyg.* **16**, 32-84.

Taliaferro, W. H. 1943. Antigen-antibody reactions in immunity to metazoan parasites. *Proc. Inst. Med. Chicago* **14**, 358-368.

Taliaferro, W. H. 1948. The inhibition of reproduction of parasites by immune factors. *Bacteriol. Rev.* **12**, 1-17.

Taliaferro, W. H. 1955. Studies in antibody formation. In: *Some Physiological Aspects and Consequences of Parasitism.* Ed. W. H. Cole, Rutgers University Press, New Brunswick, New Jersey. pp. 50-75.

Taliaferro, W. H. 1956. Functions of the spleen in immunity. *Amer. J. Trop. Med. & Hyg.* **5**, 391-410.

Taliaferro, W. H. 1957. General introduction: synthesis and degradation of antibody. In: The Synthesis and Activities of Antibody. *J. Cell and Comp. Physiol.* **50**, Suppl. 1, 1-26.

Taliaferro, W. H. and Sarles, M. P. 1939. The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with *Nippostrongylus muris. J. Infect. Dis.* **64**, 157-192.

Taliaferro, W. H. and Sarles, M. P. 1942. The histopathology of the skin, lungs and intestine of rats during passive immunity to *Nippostrongylus muris. J. Infect. Dis.* **71**, 69-82.

Taliaferro, W. H. and Taliaferro, L. G. 1950. The dynamics of hemolysin formation in intact and splenectomized rabbits. *J. Infect. Dis.* **87**, 37-62.

Taliaferro, W. H. and Taliaferro, L. G. 1951*a*. Effect of X-rays on immunity: a review. *J. Immunol.* **66**, 181-212.

Taliaferro, W. H. and Taliaferro, L. G. 1951*b*. The role of the spleen in hemolysin production in rabbits receiving multiple antigen injections. *J. Infect. Dis.* **89**, 143-168.

Taliaferro, W. H. and Taliaferro, L. G. 1952. The role of the spleen and the dynamics of hemolysin production in homologous anamnesis. *J. Infect. Dis.* **90**, 205-232.

Taliaferro, W. H. and Taliaferro, L. G. 1954. Further studies on the radiosensitive stages in hemolysin formation. *J. Infect. Dis.* **95**, 134-141.

Taliaferro, W. H. and Taliaferro, L. G. 1956. X-ray effects on hemolysin formation in rabbits with the spleen shielded or irradiated. *J. Infect. Dis.* **99**, 109-128.

Taliaferro, W. H. and Taliaferro, L. G. 1957. Amino acid incorporation into precipitin at different stages in the secondary response to bovine serum albumin. *J. Infect. Dis.* **101**, 252-274.

Taliaferro, W. H., Taliaferro, L. G. and Janssen, E. F. 1952. The localization of X-ray injury to the initial phases of antibody response. *J. Infect. Dis.* **91**, 105-124.

Taliaferro, W. H., Taliaferro, L. G. and Pizzi, A. 1958. Avidity anr the intercellular transfer of hemolysin. *Fed. Proc.:* In press.

Taliaferro, W. H. and Talmage, D. W. 1955. Absence of amino acid incorporation into antibody during the induction period. *J. Infect. Dis.* **97**, 88-98.

Taliaferro, W. H. and Talmage, D. W. 1956. Antibodies in the rabbit with different rates of metabolic decay. *J. Infect. Dis.* **99**, 21-33.

Talmage, D. W. 1957*a*. The primary equilibrium between antigen and antibody. In: The role of I^{131}-labeled proteins in biology and medicine. *Ann. New York Acad. Sci.* **70**, 82-93.

Talmage, D. W. 1957*b*. Allergy and immunology. *Ann. Rev. Med.* **8**, 239-256.

Talmage, D. W. and Freter, G. G. 1956. The blocking of labeled antibody uptake as a measure of the relative combining capacity of anti-red cell serum. *J. Infect. Dis.* **98**, 277-292.

Talmage, D. W., Freter, G. G. and Taliaferro, W. H. 1956*a*. The effect of repeated injections of sheep red cells on the hemolytic and combining capacities of rabbit antiserums. *J. Infect. Dis.* **98**, 293-299.

Talmage, D. W., Freter, G. G. and Taliaferro, W. H. 1956*b*. Two antibodies of related specificity but different efficiency separated by centrifugation. *J. Infect. Dis.* **98**, 300-305.

Talmage, D. W., Freter, G. G. and Thomson, A. 1956. The effect of whole body X radiation on the specific anamnestic response in the rabbit. *J. Infect. Dis.* **99**, 246-252.

Talmage, D. W. and Maurer, P. H. 1953. I^{131}-labeled antigen precipitation as a measure of quantity and quality of antibody. *J. Infect. Dis.* **92**, 288-300.

Terry, R. J. 1957. Antibody against *Trypanosoma vivax* present in normal cotton rat serum. *Exp. Parasitol.* **6**, 404-411.

Thompson, R. C. and Ballou, J. E. 1956. Studies of metabolic turnover with tritium as a tracer. V. The predominantly non-dynamic state of body constituents in the rat. *J. Biol. Chem.* **223**, 795-809.

Weinrach, R. S., Lai, M. and Talmage, D. W. 1958. The relation between hemolysin concentration and hemolytic rate as measured with Cr^{51}-labeled cells. *J. Infect. Dis.* **102**, 60-73.

Weinrach, R. S. and Talmage, D. W. 1958. The role of antibody in immune hemolysis. *J. Infect. Dis.* **102**, 74-80.

Whipple, C. H. 1956. *The Dynamic Equilibrium of Body Proteins.* 68 pp. Charles C Thomas, Springfield, Illinois.

Zinsser, H. 1931. *Resistance to Infectious Diseases.* 4th Ed. Macmillan, New York
(see page 136).

IMMUNOLOGICAL AND PATHOLOGICAL PHENOMENA RELATED TO SUBSTANCES FROM TISSUES OF *ASCARIS LUMBRICOIDES**

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NUMEROUS investigators have studied the reactions observed in experimental animals following inoculations of extracts from various animal parasites. The reports deal chiefly with the intravenous injection of extracts or suspensions of whole parasites and a description of resulting symptoms, but with little or no emphasis on the mechanism of the ensuing toxic reactions.

In the helminthiases, excretions and secretions from living parasites and substances from parasitic forms which disintegrate in the tissues are being constantly absorbed into the blood of the host. The similarities or differences between toxic reactions produced by metabolic products and by toxic substances from dead worms, is a topic which also has been superficially studied.

Studies performed by inoculating extracts from the tissues of *Ascaris lumbricoides var suum*, i.e., cuticle, muscle, intestine, eggs, sperm, and coelomic fluid, into experimental animals, have revealed that all of these materials contain a substance which produces fundamentally the same reaction in each animal species, and that the mechanism of the toxic reaction is apparently the same. The results also suggest that the toxic substance within the tissues of the parasite are also present in its metabolic products.

Materials and Methods

Preparation of extracts from Ascaris *tissues and tests for serum agglutinins.* Adult *Ascaris lumbricoides* var. *suum* were obtained in fresh condition and the tissues were dissected as described in a previous report

* These investigations were supported by Grant-In-Aid No. E-839, from the National Institutes of Health, U. S. Public Health Service, Bethesda, Maryland.

(Oliver-González, 1943). Each tissue was washed in distilled water and stored in the freezer at −20°C to −25°C, until ready for use; the material for extraction was suspended in 0.04 molar tris-hydroxymethyl amino-ethane buffer, and homogenized in a 100 ml. all-glass electric-run tissue grinder. The homogenate was centrifuged at 3,000 r.p.m. for 15 min. and the centrifugate was discarded. Sodium chloride was then added to make a 0.5 per cent solution, followed by the addition of 5 to 7 volumes of 95 per cent alcohol. A precipitate was recovered after storage overnight at 6°C, which was dried by various washings in alcohol and ether. The dry material was weighed and suspended in 0.85 per cent salt solution for injection.

The effect of the tissue extracts on the α_2 isoagglutinins in human serum of Groups B and O was determined by adding known amounts of the substance to small volumes of the serum. The serum-extract mixture, in 10 × 60 mm tubes was incubated at 37°C for one hour and tested with human cells of Group A_2 in order to detect the α_2 agglutinin titer. Untreated serums, diluted with saline to the same volume of the treated serums, were used as controls. The titers were observed under the stereoscopic microscope at a magnification of ×20.

Animals injected with the ascaris tissue extracts were tested for agglutinins against their own erythrocytes. One tenth ml. volume of the undiluted and of serial dilutions of the plasma were incubated with 0.05 ml. of a one per cent suspension of the erythrocytes. The plasma-erythrocyte suspension was incubated for 1 hour at 37°C and then read under the stereoscopic microscope as above.

Animal Inoculations

All inoculations of dogs were done intravenously, using the veins of the front legs. The required amount of extract was suspended in 10 ml. of saline and homogenized in an all-glass grinder.

Dogs were weighed and blood samples were obtained before inoculation and immediately after death. Complete blood and platelet counts, observations on the coagulation time, and serological tests for agglutinins were performed on all blood samples.

The animals were carefully observed after injection for reactions, and autopsies were done on as many dogs receiving each tissue extract as possible. Portions of heart, lungs, spleen, liver, kidneys and mesenteric lymph nodes were fixed in Zenker-formol fixative for histopathological studies.

Guinea pigs of both sexes weighing from 450 to 650 gm. were bled from the heart and the serum was tested for α_2 agglutinins. Using a 1 ml.

syringe and 22-gauge needle, the inoculations were made intracardially, after suspending the required amount in 1 ml. of saline. One group of normal animals was inoculated with each of the tissue extracts. Another group of guinea pigs was fed with 10,000 to 15,000 infective ascaris eggs. Ten to 15 days after feeding, the animals were injected intracardially with the ascaris tissue extracts. The guinea pigs in both groups were observed carefully for reactions, and were killed 24 hours after inoculation. Tissue studies were made as with the dogs.

The mice utilized in this study were of an A strain weighing from 22 to 30 gm. The tail vein was used for inoculations of the tissue extracts suspended in 1 ml. of saline. One group of normal mice was inoculated with each of the ascaris tissue extracts. Another group was immunized with human erythrocytes of Group A_2. Each animal received 1 ml. of a 5 per cent suspension of cells in saline, intraperitoneally, for 3 consecutive days in a week, and after a rest period of 5 days, the injections of A_2 erythrocytes were continued for another 3 days. Ten days after the last immunizing dose, mice were injected intravenously with the required dose of tissue extract. The mice in both groups were observed for reactions, and were killed 24 hours after injection of the tissue extract. Blood was collected, and the serum tested for α_2 isoagglutinins.

Normal mice injected with A_2 erythrocytes only and with tissue extract only were also killed as controls. Tissue studies were performed as with the dogs and guinea pigs.

Results

Toxic reactions and pathological changes observed in dogs inoculated with extracts from ascaris tissues. All dogs inoculated intravenously with the ascaris tissue extracts had α_2 agglutinins in the serums. The titers varied from 1:32 to 1:128. Immediately after inoculation, the animals exhibited symptoms similar to anaphylaxis. These were chiefly vomiting, urination, defecation and difficult breathing, followed by collapse and death. Animals usually died within the first hour after inoculation. The minimum lethal doses required to cause death are presented in Table I. Animals which received a dose less than that became resistant to further inoculations. It is evident from Table I that all of the tissues contain the substance which provoked the anaphylactoid symptoms and death.

The most notable postmortem findings were intense congestion of the liver and intestinal blood vessels as seen from both the mucosal and serosal aspects. Microscopically, in some organs the erythrocytes within blood vessels were agglutinated, with loss of their usual shape and sharpness of outline. This was particularly extensive in the liver, which showed

intense congestion. The spleen showed moderate congestion and depletion of lymphocytes in the follicles. Pathological studies on these and other tissues will be considered in greater detail in another report.

Hematological studies revealed a marked drop in platelets and white blood cells. The erythrocytes increased, probably as a result of hemoconcentration due to the loss of liquids. The blood lost its coagulability indefinitely.

The plasma of inoculated dogs agglutinated the animal's own erythrocytes. Agglutination was observed at low titers, after incubation for 1 hour

TABLE I

MINIMUM AMOUNT OF EXTRACT FROM TISSUES OF *Ascaris lumbricoides var suum* CAUSING DEATH OF DOGS WITH α_2 AGGLUTININS IN THE SERUM

Number of Dogs	Tissue Extract Inoculated	Average Mgm/Kilogram	Reactions	
			Anaphylactoid and Death	Main Pathological Findings
8	Cuticle	0.3	Positive	Liver Congestion
6	Cuticle	0.5	Positive	Liver Congestion
7	Cuticle	1.0	Positive	Liver Congestion
3	Egg	0.5	Positive	Liver Congestion
4	Egg	1.0	Positive	Liver Congestion
2	Intestine	18.0	Positive	Liver Congestion
2	Intestine	15.0	Positive	Liver Congestion

(Table II) at 37°C, but tended to disappear after prolonged incubation for longer periods of time.

Observations on Guinea Pigs and Mice. Normal guinea pigs exhibited no symptoms after inoculation with each of the tissue extracts. Such manifestations as scratching of the nose, coughing or gasping for breath, which are readily observable in sensitized guinea pigs, did not appear in these animals. However, the histopathological studies revealed that the guinea pigs in both groups of animals which had a high titer of α_2 isoagglutinins in the serum, had developed as marked a congestion of the liver as was observed in the dogs (Table III).

No anaphylactoid reactions appeared in 19 guinea pigs infected with ascaris 10 to 15 days previously, and intracardially injected with 5 to 15 mg/kg of extract of ascaris cuticle. These animals at autopsy had numerous ascaris larvae in the lungs.

No anaphylactoid reactions were observed in any of the mice of the various groups, i.e., those immunized against A_2 erythrocytes, those injected intravenously with the tissue extracts, and the ones injected with

TABLE II

AUTOAGGLUTININS AGAINST DOG'S OWN ERYTHROCYTES AFTER INTRAVENOUS INOCULATION WITH EXTRACTS FROM *Ascaris* TISSUES CONTAINING THE α_2 INHIBITING SUBSTANCE

Dog Number	Tissue Extract Inoculated	Before Inoculation							After Inoculation, at the Time of Death						
		Undiluted Plasma	Dilutions						Undiluted Plasma	Dilutions					
			2	4	8	16	32	64		2	4	8	16	32	64
524	Cuticle*	Negative							3+	2+	+	+	0	0	0
525	Cuticle	Negative							4+	2+	+	+	+	+	0
535	Cuticle	Negative							2+	+	+	0	0	0	0

* Results of tests in plasma of dogs inoculated with extracts of egg, intestine, and sperm were the same as those from dogs inoculated with cuticular extract.

the tissue extracts after immunization with A_2 erythrocytes (Table IV). These animals behaved as the non-sensitized controls.

Marked differences were observed, however, in the tissues from the animals immunized against human Group A_2 erythrocytes, and then challenged with the tissue extracts, as compared with the liver sections from the animals injected with each of the other above substances. No congestion or agglutinated corpuscles were observed in the mice immunized against A_2 erythrocytes or those injected intravenously with ascaris tissue extracts, but intense congestion of the liver, with large coagulated

TABLE III

INTRACARDIAC INOCULATION OF THE EXTRACT FROM *Ascaris* CUTICLE INTO NORMAL GUINEA PIGS*

Number of Guinea Pigs	Titer Against A_2 Erythrocytes Before Inoculation	Mgm/Kilogram	Reactions	
			Anaphylactoid and Death	Histological Liver
6	Negative	10	No	Normal
6	Negative	15	No	Normal
5	Negative	20	No	Normal
4	1:4	20	No	Normal
6	1:8	20	No	Congestion
6	1:32	20	No	Congestion

* Similar results were obtained with the extracts from the other ascaris tissue extracts.

masses of erythrocytes, was observed in those mice in which agglutinins had been developed in the serum and which were then injected with ascaris extracts.

DISCUSSION

The fatal reactions produced in dogs after inoculation of each of the ascaris tissue extracts do not differ in any way from the reactions previously described by Rocha e Silva and Graña (1946). The substance used by these investigators was an extract from the whole adult worm described as mainly nitrogenous, and as a proteose of high molecular weight. The symptoms and tissue changes observed in both studies were indistinguishable from that observed in anaphylactic shock. Clinically, there is vomiting, urination, defecation and collapse. Pathologically, engorgement of the liver, decreased blood coagulation, diminished platelets, and leukopenia were observed constantly. In our study, the substance which causes the above reaction is present in varying amounts in all ascaris tissues and is easily extracted with a buffer at pH 8.9. The buffer used in this study (0.04 molar-tris-hydroxymethyl aminoethane) does not affect

TABLE IV

INTRAVENOUS INOCULATION OF THE *Ascaris* CUTICLE EXTRACT INTO NORMAL MICE AND MICE PREVIOUSLY IMMUNIZED AGAINST HUMAN GROUP A_2 ERYTHROCYTES

Normal					Inoculated with A_2 Erythrocytes			
			Reactions				Reactio ns	
Number of Mice	Titer Against A_2 Erythro-cytes	Mgm/ Kilogram	Anaphylac-toid and Death	Histological Liver	Number of Mice	Titer Against A_2 Erythrocytes	Mgm/ Kilogram	Anaphylac-toid and Death	Histological Liver
5	Negative	25	No	Normal	3	1:32 to 1:64	25	No	Congestion
5	Negative	50	No	Normal	5	1:16 to 1:64	50	No	Congestion
5	Negative	50	No	Normal	5	1:16 to 1:64	50	No	Congestion

tissue enzymes, and will extract water soluble substances.

The substance isolated from the ascaris tissue is by no means a pure entity. Investigations are under way to isolate the active principle and thus perform chemical studies.

The substance which causes fatal reactions in dogs is shown to be related to the A_2 isoagglutinogen in human Group A_2 erythrocytes, by the fact that when added to human serums of Groups O and B, it inhibits the α_2 isoagglutinins in the serum. Each of the ascaris tissue extracts has been found to posses such property (Table V). The secretions and excre-

TABLE V

MINIMUM AMOUNTS OF EXTRACTS FROM *Ascaris* TISSUES REQUIRED TO INHIBIT THE α_2 ANTIBODY IN HUMAN SERUM OF GROUPS B AND O

Tissue from Which Substance Was Extracted	Milligrams of Substance per cc of Serum	α_2 Titers Before and After Adding Substance			
		Group B Serum		Group O Serum	
		Before	After	Before	After
Cuticle	1.0	1:32	0	1:64	0
Egg	3.0	1:32	0	1:32	0
Coelomic Fluid	1.3	1:64	0	1:32	0
Intestine	1.0	1:16	0	1:64	0
Sperm	2.1	1:64	0	1:32	0

tions of animal parasites and bacteria also inhibit the α_2 isoagglutinins in human serums (Oliver-González, 1954). This suggests that the A_2 isoagglutinogen-like substance is of metabolic importance to the infecting parasites, since it is found in all tissues of the worms, and it is present in the metabolic products.

There is some evidence suggesting that one of the factors responsible for the death of the dogs is intravascular agglutination of erythrocytes. In the first place, plasma of animals inoculated with the tissue extracts agglutinated the animal's own erythrocytes. This did not occur before inoculation. Secondly, the sections of liver and other organs showed agglutination of erythrocytes in large clumps. Rocha e Silva and Porto (1946) reported agglutinated platelets and leukocytes in their animals that died after the intravenous injection of ascaris extracts, but they make no mention of agglutinated erythrocytes. Agglutination of erythrocytes, as well as of platelets and leukocytes, is therefore an important finding in the possible mechanism of death.

From the above studies it is not definitely established what the antigen and the antibody are that react to cause agglutination. Apparently the

α_2 isoagglutinins normally present in the animal sera constitute the antibody and the A_2 isoagglutinogen-like substance present in the ascaris tissue extract constitutes the antigen. It has to be postulated, therefore, that the A_2 substance from the worms adheres to the erythrocytes and that the agglutinin reacts with the red cells which have the homologous agglutinogen on their surfaces. The above studies merely suggest that such may be the case, but lack evidence to show that the substance introduced goes totally or in part to coat the erythrocytes. Finer techniques of experimentation are necessary, particularly tagging of the substance with radioisotopes which would facilitate following the fate of this substance in the animal's body.

Other indirect evidence suggests that the α_2 isoagglutinins play a major role in the agglutination of erythrocytes. Intravenous inoculation of normal mice with the α_2 agglutinin-inhibiting substance present in the ascaris tissue extracts cause no congestion of the liver. However, the liver of mice immunized with human A_2 erythrocytes, and therefore with the α_2 agglutinins in the serum, showed marked congestion of the liver after injection of the tissue extracts. Therefore, as in the dogs, the α_2 agglutinins in the serum and the A_2 substance introduced through inoculation were requisites for the reaction.

The infection of guinea pigs with ascaris, which is manifested by the presence of larvae in the lungs, apparently did not sensitize the animals to the tissue extracts. Although the metabolic products of these larvae may constitute a source of antigen to stimulate the formation of α_2 antibodies, these antibodies could not be detected in the serum. This may have been due to the fact that the antigenic stimulus was inadequate. Although numerous larvae were present in the lungs, their stay in this organ is short, since they migrate into the intestine and are rapidly expelled. The reactions observed in the dogs, guinea pigs and mice are, therefore, not due to previous sensitization with ascaris larvae, but to some other mechanism such as explained above.

As has been shown in previous studies, the A_2 isoagglutinogen-like factor of infectious agents does not inhibit the α_1 or β agglutinins in human serums, as tested *in vitro* (Oliver-Gonzalez, 1954). Mice should be immunized with human erythrocytes of Groups A_1, B and O, and then tested by injection with the ascaris tissue extracts. The *in vivo* reaction may not be specific to the A_2 erythrocytes.

The tissues from ascaris contain other antigenic substances which upon injection into rabbits lead to the development of antibodies related to other types of immunological reactions (Oliver-Gonzalez, 1943), but in the studies here presented we are dealing with a factor which is common

to all tissues, but which is responsible for one type of reaction. (There are, however, other substances in the tissues responsible for other types of antigen-antibody reactions.)

Summary

1. Extracts were prepared from cuticle, muscle, intestine, egg, sperm, and coelomic fluid dissected from adult *Ascaris lumbricoides* var. *suum*. Each material was extracted in 0.04 molar tris-hydroxymethyl-aminoethane, and the soluble substance was then precipitated with 95 per cent alcohol.

2. Each extract, when injected intravenously into dogs, caused an anaphylactoid type of reaction followed by rapid death. The main pathological findings were congestion of the liver and intestinal veins. There was a marked decrease in leukocytes and platelets, and incoagulability of the blood.

3. Histopathological studies showed agglutination of erythrocytes, observed particularly in sections of the liver. The blood plasma of the inoculated dogs agglutinated the animal's own erythrocytes.

4. Guinea pigs inoculated intracardially with the ascaris tissue extracts did not exhibit anaphylactoid symptoms, but marked congestion of the liver was seen in the animals with a high α_2 isoagglutinin titer in the serum. No congestion of the liver was observed in the guinea pigs without α_2 antibodies.

5. Guinea pigs infected with ascaris by feeding embryonated eggs prior to inoculation of the tissue extracts did not respond with the anaphylactoid reaction. This suggests that such a reaction is not related to previous sensitization with migrating ascaris larvae.

6. It is suggested that, among other mechanisms, autoagglutination of the erythrocytes may have an important role in the reactions of animals inoculated with the ascaris tissue extracts; also, that autoagglutination is mediated by α_2 isoagglutinins, normally present in the animals sera, which react with the injected antigen. It is also postulated that the antigen, when injected, adheres to the erythrocytes.

REFERENCES

Oliver-González, J. 1943. Antigenic analysis of isolated tissues and body fluids of roundworm *Ascaris lumbricoides* var. *suum*. *J. Inf. Dis.* 72, 202.

Oliver-González, J. 1954. Immunological properties of polysaccharides from animal parasites. *Ann. Rev. Microbiol.* 8, 353-362.

Rocha e Silva, M., and Graña, A. 1946. Anaphylaxis-like reactions produced by ascaris extracts. *Archives of Surgery* 52, 523-537.

Rocha e Silva, M., Graña, A., and Andrade, Sylvia O. 1946. Anaphylaxis-like reactions produced by ascaris extracts. *Archives of Surgery* 53, 199-213.

CONTRIBUTIONS TO THE IMMUNOLOGY AND SEROLOGY OF SCHISTOSOMIASIS

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THE superfamily Schistosomatoidea (Stiles & Hassall, 1926: LaRue, 1957) comprises a large group of trematodes with species parasitizing many classes of the phylum Chordata. What were formerly thought to be three distinct human species, *Schistosoma haematobium, Schistosoma mansoni* and *Schistosoma japonicum,* are believed today to be complexes of closely related species or varieties which infect man and lower animals (Kuntz, 1955). With the incidence of malaria decreasing in the world, schistosomiasis is becoming one of the more important parasitic diseases of the human race. Millions of people are infected in the Middle East, the Orient, Africa, and South America. The economic toll and loss of productivity which schistosomiasis causes in these areas of the world makes it important that we study and understand the biology, physiology, epidemiology and host-parasite relationships of these parasites. Armed with this knowledge we may hope to establish effective control procedures to eradicate or alleviate manifestations of this disease.

In recent years advances made in the chemotherapy of schistosomiasis make it important the immunology of the disease be better understood. Increasingly larger groups of people are being treated in endemic areas and the effect of treatment on resistance and immunity toward this infection has not been thoroughly evaluated. To this day, the mechanisms of immunity against schistosomiasis in man and animals are still obscure. In this paper contributions made on the immunology and serology of schistosomiasis by the author and his co-workers will be reviewed. In an effort to discuss all aspects of the subject, papers by other workers will be cited.

It is not feasible to list all pertinent references relating to the immunology and serology of schistosomiasis. The number of published works is quite large. In general, recent references are cited and no attempt at completeness has been made. Two bibliographies list references up to 1948 (Khalil, 1931 and Bouillon, 1950). General reviews by Fairley (1951) and Newsome (1956) and books and monographs by Gelfand (1950), Skrjabin (1951), Girges (1934), and Martins (1949) plus many special reviews are available for reference.

In our laboratory we have been interested in the immunology and serology of *Schistosomatium douthitti* (Cort, 1914), a natural parasite of meadow voles (*Microtus pennsylvanicus*), and muskrats (*Ondatra zibethica*). This schistosome is endemic in the United States. We have also studied *S. mansoni*, a human species. Our *S. mansoni* strain was originally from Puerto Rico* and has been maintained by serial passage through laboratory reared *Australorbis glabratus* and hamsters. The infection with *S. douthitti* was obtained from naturally infected *Lymnaea palustris* collected in Ann Arbor, Michigan, and Chicago, Illinois, and maintained by serial passage through *L. palustris* and hamsters (Kagan, Short and Nez, 1954).

Our immunological studies were made with white mice, *Mus musculus*, and rhesus monkeys, *Macaca mulatta*. We have investigated various facets of the host-parasite relationship of *S. douthitti* because we believe there is fundamental value in studying the immunology of a near natural host-parasite relationship. We also believe that studies on animal schistosome species may bear directly on human species. In many respects *S. douthitti* appears to be related to *S. japonicum*. In our studies on chemotherapy with Miracil D (Kagan & Lee, 1952), we observed that *S. douthitti* was completely resistant to the action of this drug. Pesigan (1951) reported similar results with *S. japonicum*. These two species have eggs similar in size and shape which differ markedly from those of *S. mansoni* and *S. haematobium*. The nocturnal emergence of cercariae from the snail host and the behavior of the cercariae in clinging to the surface film of the water awaiting penetration of the mammalian host is identical for both species. The gross pathology in mice and rate of infection for mice for these two species are almost the same. These facts, plus the reported occurrence of *S. japonicum* in rodents (Hsü & Li, [in press], Magath & Mathieson, 1945), led us to believe that perhaps *S. japonicum* was a rodent schistosome species with broad host specificities (Kagan & Lee, 1952).

The presence of antibodies in infected or antigen-injected animals has been recognized in schistosomiasis for many years. The terms immunology and serology are used quite loosely and, in the minds of some, are synonymous. In this review serology will refer to antigen-antibody reactions whereas immunology will imply resistance to infection. Thus a host may manifest a serological response (the production of antibodies) in the absence of an immune response (resistance or protection). In many instances, but not in every case, the development of resistance is mediated through the action of an antigen-antibody reaction. The emphasis on the

* The original stock of infected snails was obtained in 1950 from the Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service.

former circumstance (presence of antibodies) may account for the fact that the two terms are not always used correctly.

The mechanisms of helminth immunity for many related species involve both humoral and cellular factors (Taliaferro, 1950). To demonstrate a humoral antibody mechanism, three criteria have to be met: (1) antibodies must be present in the serum of the immune host; (2) the serum of the immune host on passive transfer to a normal animal must be protective; and (3) the protective antibody should be removed from the serum by absorption with the parasite (somatic antigens) or antigens produced by the living parasite (metabolic products antigen).

Cellular host factors in immunity may involve an enhanced allergic or phagocytic response or hyperplasia of the reticulo-endothelial system. These manifestations may, under certain circumstances, lead to the death, encapsulation, or isolation of the parasite, and/or the production of antibodies. In addition to the above humoral and cellular mechanisms, there may be non-specific defense mechanisms which do not require the mediation of antibodies or cells.

The following immune states are generally recognized and the discussion of immunity will follow this general outline (Boyd, 1956).

I. Innate Immunity (constitutional or racial)
II. Acquired Immunity
    1. Active —a. Infection (natural)
                b. Vaccination (artificial)
    2. Passive—c. Natural (congenital)
                d. Artificial (serum transfer)

The serology of schistosomiasis encompasses the detection of antibodies in the serum of an infected host by both *in vitro* and *in vivo* methods. Such standard serological tests as complement-fixation, precipitin, flocculation and hemagglutination, have been used in diagnosis. *In vitro* studies of serum antibodies on larval and adult schistosome stages have been made. These studies have characterized the CHR antibody, (Cercarienhüllen reaction of Vogel & Minning, 1949*a*, 1949*b*), miracidial immobilization test (Senterfit 1953), circumoval precipitin test (Oliver-Gonzalez, 1954) and cercarial agglutination test (Liu & Bang, 1950) (Fig. 1). *In vivo* serological studies include the Prausnitz-Küstner reaction and the skin test.

Innate Immunity

Native or innate resistance encompasses the hereditary defense mechanisms possessed by a species against infectious organisms. Susceptibility studies of host species for laboratory infections with schistosomes are in one sense investigations of innate resistance against infections (Moore,

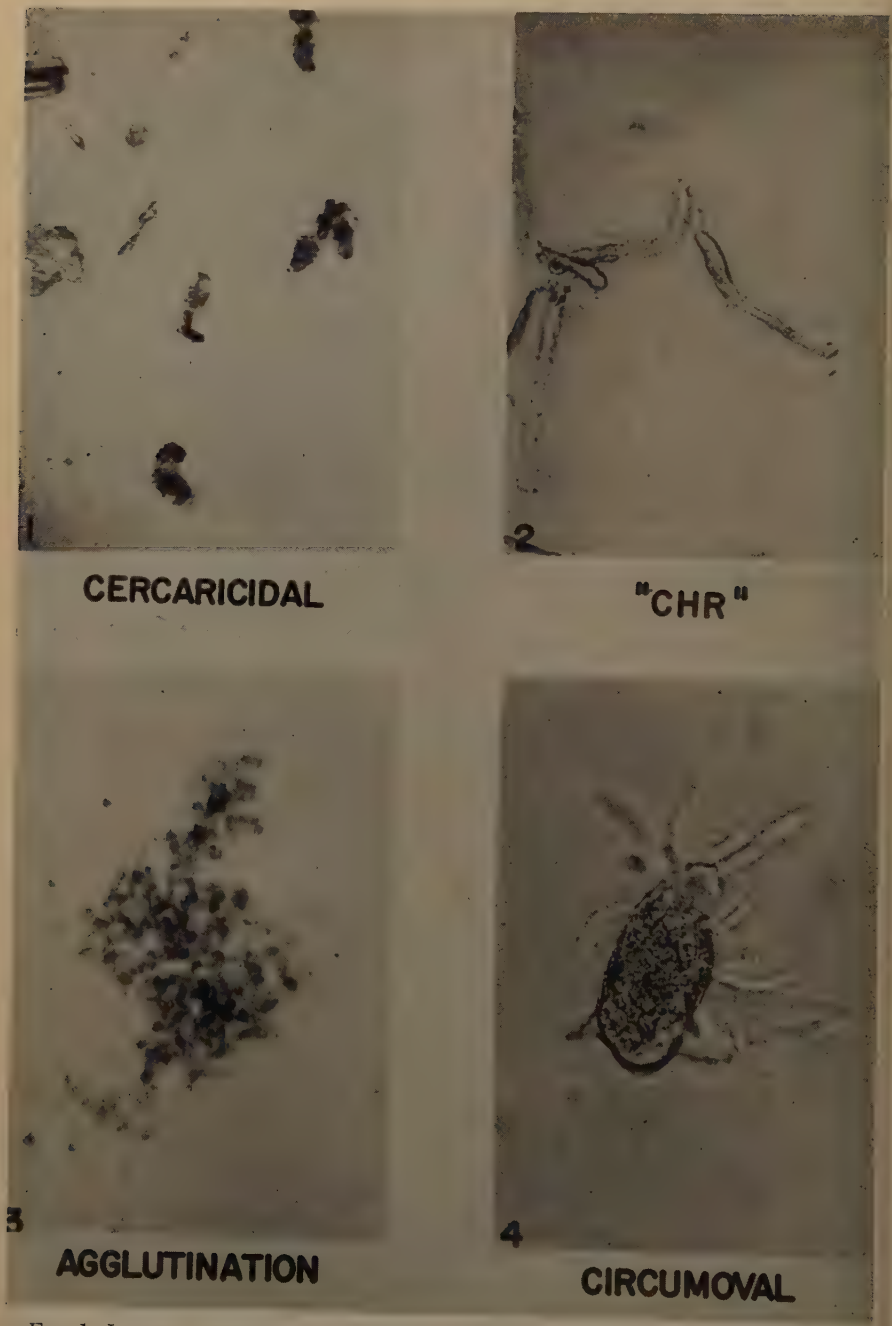

FIG. 1. *In vitro* reactions with eggs and cercariae of *Schistosoma mansoni*. Cercaricidal precipitins (Papirmeister & Bang, 1948), CHR (Vogel & Minning, 1949), agglutination (Liu & Bang, 1950) and circumoval precipitin reaction (Oliver-Gonzalez, 1954).

Yolles & Meleney, 1949; Stirewalt, Kuntz and Evans, 1951; Büttner, 1953; and Kuntz, Malakatis & Wells, 1955). As reviewed by Kuntz (1955) data from a number of workers indicate that the human schistosomes in Africa and South America are not as host specific as once supposed. *S. mansoni*, once thought to be restricted to primates, has been reported from wild rodents, insectivores and opossums (Martins, *et al.*, 1955; Schwetz, 1955).

Animal species can be divided into three categories with regard to infection with schistosomes: those that are totally resistant to infection, those that can be infected but are poor hosts, and those that are susceptible hosts.

We have infected mice, hamsters, rats, rabbits and monkeys in our laboratory with *S. douthitti*. The host-parasite relationship in these animals differs greatly. The monkey will tolerate an infection for 2-3 weeks, at which time the worms are suddenly killed and infection dramatically terminated. We found living worms in 9 monkeys 10-25 days after exposure, dead and moribund worms in 2 monkeys 22-25 days after exposure, and no worms in 5 monkeys necropsied 30-90 days after exposure (Kagan, 1953). A similar phenomenon was reported by Fairley, *et al.* (1927, 1930) for *S. spindalis* in *Macacus sinicus*. They found that monkeys remained infected for only 11-15 days. Spontaneous cure in monkeys infected with *S. mansoni* has also been reported (Cram & Files, 1947).

In the rat the termination of infection is not so dramatic. The rat is a poor host for *S. douthitti* (Price, 1931). It has been our experience that worms recovered from rats are smaller than worms from mice and hamsters. The cellular response in the rat is very extensive, with encapsulation and nodule formation occurring in the lungs of the animal early in the infection (Kagan and Meranze, 1957). The rat is also a resistant host for *S. mansoni*, and spontaneous cure after 6-8 weeks has been reported, (Stirewalt, Kuntz and Evans, 1951; Thompson, 1954). The rabbit is another poor host for *S. douthitti*. Massive doses of cercariae resulted in light infections.

The mouse and hamsters are very susceptible hosts for *S. douthitti* infections. Infections lasting 468-484 days in mice were reported by Short (1952), and a male infection of 828 days was reported in our work from an experimentally infected deer mouse, *Peromyscus maniculatus* (Kagan, Short and Nez, 1954). In the mouse the life span of the worms, once maturity is reached and when the infection is not too heavy, is often (possibly in most cases) limited by the death of the host. A similar situation may exist in man. Infections of over 20 years with *S. haematobium* have been reported (Berberian, *et al.*, 1953). In a survey of the incidence of *S. mansoni* in the Puerto Rican population, Ferguson *et al.* (in press) reported a relatively stable level of infection in the adult popu-

lation (Figure 2). The pleateau in the incidence curve after age 30 implies an infection of long duration. The possibility of reinfection in the female population cannot be ruled out since this is an endemic area. Rural women come in contact with infected waters in the washing of clothes and other household chores and may be continuously exposed. This is not true for the male population once maturity is reached. The incidence of infection in non-endemic parts of the island is very similar.

The schistosome cercariae are very invasive organisms. The skin is not

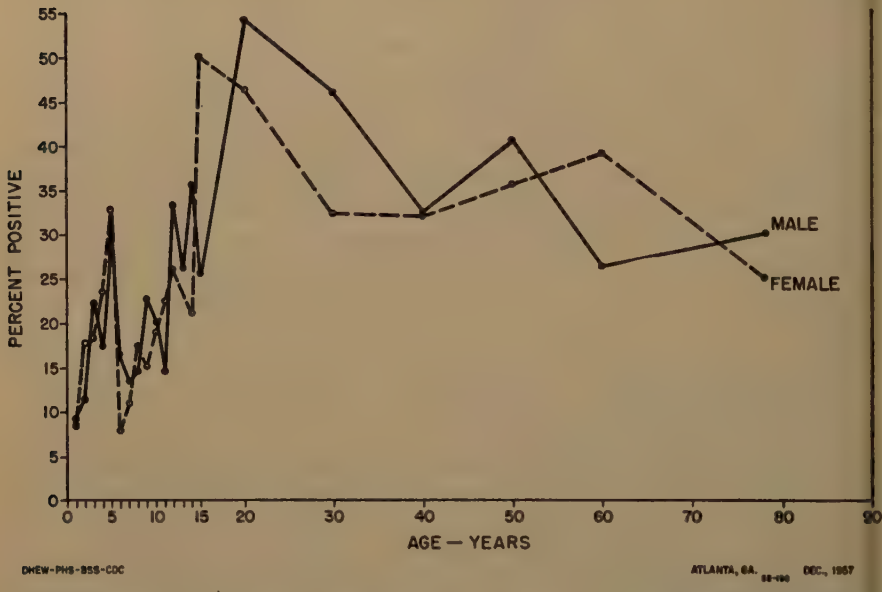

FIG. 2. Incidence of infection with *Schistosoma mansoni* in 5402 stool samples in an endemic area in Puerto Rico. (Ferguson, F. F., *et al.*, in press.)

as effective a barrier as was previously thought. It has been demonstrated that non-mammalian schistosome cercariae will penetrate mammalian hosts and migrate to the lungs before death (Olivier, 1953). The factor that causes the death of cercariae in unsuitable hosts is probably associated with the innate immunity of the host.

A number of workers have investigated the *in vitro* activity of cercariae in normal serum and have demonstrated a cercaricidal factor (Tubangui and Masilungan, 1936; Culbertson & Talbot, 1935; Culbertson, 1936; Standen, 1952; Stirewalt and Evans, 1955; Kagan and Levine, 1956). In our laboratory we were interested in determining if there was a correlation between the cercaricidal activity of the normal serum of resistant and

susceptible hosts. We bathed cercariae of *S. monsoni* in the serum of 18 animal species and noted that some sera were cercaricidal, others inhibited the locomotion of the cercariae, and two agglutinated cercariae, Table 1, (Kagan & Levine, 1956). We found that the sera of hosts with a high degree of resistance to infection with *S. mansoni* were more cercaricidal than the sera of hosts susceptible to infection. We also found that when the cercaricidal activity was eliminated by heat, the sera of additional species agglutinated cercariae (Standen, 1952; Kagan & Levine,

TABLE 1

In vitro ACTIVITY OF CERCARIAE OF *Schistosoma mansoni* IN SERA OF ANIMALS

Normal Sera			Normal Heated Sera	
Cercaricidal	Inhibition of Locomotion	Agglutination	Normal Swimming	Agglutination
Chicken	Cat	Cow	All species tested	Cow
Dog	Hamster	Horse		Dog (50%)
Goat	Man			Goat
Guinea pig	Mouse			Hamster (33%)
Pig	Monkey			Horse
Rat	Pigeon			Man (43%)
Sheep	Rabbit			Pig
Steer	Squirrel			Rabbit (11%)
				Sheep
				Steer

1956). The agglutinins we found in normal serum were non-specific antibodies unrelated to the agglutinins found in immune serum. Normal serum which agglutinated living cercariae would not agglutinate sensitized sheep cells in a tannic acid hemagglutination test (Kagan, 1955a).

Evans, Stirewalt and MacKenzie (1955) fractionated normal mouse serum electrophoretically and noted that the gamma and beta globulin fractions contained the cercaricidal factor. The alpha fraction inhibited motility, but the albumin fraction was without activity.

The cercaricidal factor of normal serum has been related to complement since it could be destroyed by heating for 30 minutes at 56°C (Tubangui and Masilungan, 1936; Culbertson, 1936; Standen, 1952). In our work we also thought initially that the cercaricidal activity was related to complement but later we decided it was not, because guinea pig serum frozen for 60 days and heated for 30 minutes at 56°C was still cercaricidal. Recent studies have indicated that the cercaricidal factor of normal serum is associated with the properdin level of serum (Iralu, unpublished). Properdin, a euglobulin containing lipid, carbohydrates

and phosphorus was discovered by Pillmer (1954) and is a minor constituent of normal serum (0.03%). With the cooperation of complement and magnesium ions it acts as a non-specific defense factor in the blood against all types of infectious agents.

There have been some experimental data with respect to host susceptibility by various schistosome species "strains" (Gönnert & Vogel, 1955; Büttner, 1953), and the effect of cortisone on innate immunity (Coker, 1957). There has been little work on the susceptibility of inbred strains of host for schistosome infection.

We did some preliminary work to determine if the incidence of infection to inbred strains of mice varied significantly when exposed to cercariae of S. *douthitti*. Although the number of animals was too small to be statistically significant, there appeared to be a tendency for the C_3H (57.79%) and the *dba* mouse (65.09%) to be slightly more susceptible for infection than the CFI mouse (48.84%) (Kagan, Short and Nez, 1954).

Cardoso (1953) after examining several hundred Negro and white Brazilians infected with S. *mansoni* came to the conclusion that the Negro race was more resistant to S. *mansoni* than the white race. These claims for man have to be evaluated very carefully because of the role that nutrition and general environmental conditions play in schistosome infection (Chandler, 1953; Le Grange, 1955; Smith, 1955).

Acquired Immunity

Although experimental evidence is meager, it is generally accepted that man acquires resistance for schistosomiasis (Fisher, 1934; Schwetz, 1956). We were able to demonstrate that the white mouse (*Mus musculus*) and the monkey (*Macaca mulatta*) developed an acquired immunity against S. *douthitti* (Kagan, 1952, 1953). In the mouse, resistance and immunity was manifested by a statistically significant decrease in the number of worms which developed from a challenging exposure of 50 cercariae in previously infected mice as compared to the number of worms which developed from 50 cercariae in initially uninfected mice. Immunization by repeated small exposure to cercariae of both sexes increased the resistance of mice to a challenging exposure. An initial infection with male and female worms evoked an immune response sooner than an initial infection with female worms. Infection with male worms over a 210-day period failed to protect mice from challenging infections (Fig. 3).

In addition to the fewer worms which developed in the immune animal, infection with female worms caused stunting of male worms in the challenging infection. Infection with male worms caused stunting of female worms, and initial infection with worms of both sexes caused

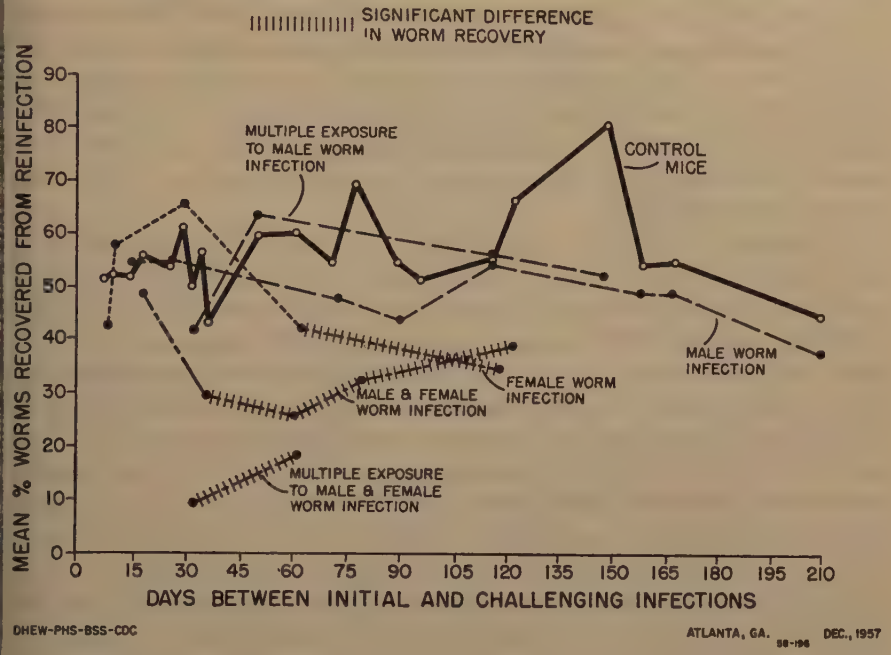

FIG. 3. Acquired immunity in mice infected with *Schistosomatium douthitti* and challenged at varying intervals of time after infection (Kagan, 1952).

stunting of all the worms in the challenging exposure (Kagan, 1952). In the immune monkey the cercariae (*S. douthitti*) of the challenging infection were eliminated before they had reached the lungs (Kagan, 1953).

Studies by Fairley (1926) for *S. spindalis,* Oliver and Schneidermann (1953), Stirewalt (1953), Pifano (1953), Thompson (1954), Laurie and de Meillon (1957), Meleney and Moore (1954) for *S. mansoni,* and Ozawa (1930), Lin, Ritchie and Hunter (1954), Vogel and Minning (1953), Hunter, *et al.,* (1956) for *S. japonicum,* all reported acquired immunity in experimental animals.

In mice infected with *S. douthitti,* the egg appears to be an important source of antigenic stimulus for the immune state. The evidence for this is primarily circumstantial. Resistance in mice infected with male and female worms or only with female worms coincided with egg deposition in the tissues by the sexually mature worm. The female *S. douthitti* can produce viable eggs hermaphroditically in the absence of male worms (Short, 1952). We found that the egg of *S. douthitti* was very resistant to chemotherapy and survived in the tissues 3-4 weeks after the death of the worms. This is also true for the longevity of the eggs of *S. mansoni*

(Gönnert, 1955) and S. *japonicum* (Vogel and Minning, 1947). Mice infected with S. *douthitti* and cured by chemotherapy lose their immunity approximately 3 weeks after the death of the worms (Kagan and Lee, 1953). This interval of time coincides with the longevity of the eggs in the tissues and suggests that perhaps the living embryo within the egg secretes or excretes substances that are necessary for the maintenance of acquired immunity in the mouse.

In addition to the eggs serving as an antigenic stimulus there is experimental evidence that in the monkey and perhaps in man, adult worms and/or their metabolic products may also be responsible for the antigenic stimulus of the immune state. Girges (1934) expressed his belief that a male infection in man was protective against superinfection. He based this statement on data obtained from the autopsy table since in chronic human infections he occasionally found only male worms. Vogel and Minning (1953) reported that monkeys infected with male S. *japonicum* worms were protected from lethal superinfections of cercariae 1-2 years after cure. Lin, Ritchie and Hunter (1954) also found that mice infected with male S. *japonicum* worms were resistant to superinfection. In our studies with rhesus monkeys, animals challenged after the initial infection had been eliminated by spontaneous cure were resistant to reinfection (Kagan, 1953).

Immunization Experiments

One of the most significant contributions in the immunology of parasitic disease has been the experimental demonstration that "metabolic antigens" play a significant role in parasitic resistance (Thorson, 1953, 1954; Thillet and Chandler, 1957). Workers with S. *japonicum* have reported some measure of success with immunization experiments with somatic or killed body antigens of adult worms. Ozawa (1930), Kawamura (1932) and Lin, Ritchie and Hunter (1954) reported that experimental animals were protected by previous injection of saline suspension of whole worms. Vogel and Minning (1953) were unsuccessful in thus immunizing monkeys against infection with S. *japonicum*. Watts (1949) reported that mice injected with a cercarial antigen of S. *mansoni* were more resistant to infection than non-injected mice. Thompson (1954) was unable to verify Watt's results; in his experiments, no protection was manifested in immunized mice. In our laboratory we conducted a series of experiments with somatic antigens of S. *douthitti* with negative results. In almost every instance antigen-injected mice were more susceptible to infection than the non-antigen injected controls. We believe this was due to their resistance being lowered by the injection of massive doses of foreign protein (Table 2).

Table 2

Table 2. Immunization Experiments with Antigens from the Life Cycle of *Schistosomatium douthitti*

Antigen	Immunizing Dose	Number of Mice		Cercariae Challenge	Number of Worms Recovered	
		Immunized	Controls		Immunized	Controls
1300 male and female adult worms	.1 ml×6	6	6	50	30.0	33.5
1200 male and female adult worms	.5 ml×3	4	4	50	20.5	20.0
100 male adult worms	.5 ml×7	8	9	50	33.0	29.0
Infected snail	.5 ml×6	8	4	50	15.1	20.0
Normal snail	.5 ml×6	8	4	50	16.8	20.0
Eggs in Liver	0.25–0.5×6	8	6	25	8.2	5.5
Eggs in Liver	0.5 ml×7	6	6	25	13.3	11.1
Eggs in Liver	0.5 ml×9	12	8	25	8.7	5.0

Recent work by my former graduate student with metabolic antigens of cercariae and adult worms of *S. mansoni* have indicated that the life of mice exposed to lethal doses of cercariae may be prolonged by immunization with these antigens (Levine, unpublished). The metabolic antigen was obtained by incubating approximately 400,000 cercariae in 5-10 ml of aquarium water at room temperature overnight. The cercariae were removed and the aquarium water containing the cercarial secretions, excretory and metabolic products was saved. This process was repeated four times using the same fluid, so that antigen from approximately 1.6 million cercariae was obtained. A similar antigen was made by substituting 300 male and female worms for each incubation period.

Five groups of mice were exposed to a lethal dose of 700 cercariae of *S. mansoni* after a period of immunization. Group A was unimmunized, group B received a sham immunization of aquarium water in Freund's adjuvant (Arlacel A plus Bayol F and BCG, Cohn, 1953), groups C, D, and E received cercarial and adult metabolic product antigen in Freund's adjuvant once each week for 5 weeks and were then exposed to 700 cercariae. Mice exposed to 700 cercariae begin to die in the 8th week due to passage of eggs through the wall of the intestine. Perhaps because of excess handling or the deleterious effect of the oil in the antigen, mice in the immunized and control group died during immunization and shortly after exposure to cercariae. In the analysis of the results, Levine took the 8th week after exposure as the start of the experiment and calculated the longevity of the immunized mice compared to the controls for all mice alive after 7 weeks of infection (Table 3). As can be seen from the

TABLE 3

IMMUNIZATION EXPERIMENTS WITH METABOLIC ANTIGENS OF *Schistosoma mansoni* (Levine, D. M., unpublished).

Group	A	B	C	D	E
No. of Mice	15	15	15	15	15
Protocol	No Vaccination	Aquarium Water	Cercarial metabolic antigen	Adult metabolic antigen	Cercarial and Adult metabolic antigen
No. of mice surviving immunization and exposure to Cercariae	10	9	12	13	11
No. of mice surviving 7 weeks of infection	10	4	8	11	8
Mean week of death for mice alive at 8 weeks of infection	10.1±1.8	10.0±1.4	12.8±3.1	11.9±2.8	12.5±2.4
Average mean week of death for control and experimentals	10.1±1.6		12.4±2.7		
Difference between means	2.3±0.67				
T value	3.4 (significant at 1% level)				

data the mean week of death for the immunized mice was significantly later than for the controls, indicating that immunization prolonged the life of these mice. If one applies the same analysis to longevity experiments reported by Olivier and Schneidermann (1953) the mean week of death for mice immunized by previous infection is also significant in some experiments.

With techniques for maintaining schistosomes *in vitro* (Robinson, 1956a; Newsome and Robinson, 1954), I believe sufficient concentrations of metabolic antigens can be obtained to evaluate the use of these antigens in the study of the immunology and serology of the disease.

PASSIVE TRANSFER

Natural (congenital): There has been to my knowledge no study on the transfer of immunity congenitally or in the colostrum. Infants and very young children are not infected with schistosomes because they do not

come in contact with infected cercariae. As soon as they are old enough to play in infected waters they probably are exposed (Ferguson, *et al.*, in press).

Artificial passive transfer: Antibodies are present in the serum of an infected animal. The nature and character of some of these antibodies will be described in detail in the serology portion of this paper. In order to demonstrate an antibody basis for schistosomal immunity passive transfer experiments should be successfully performed. Kawamura (1932) reported passive transfer in dogs and rabbits infected with *S. japonicum.* This report is difficult to evaluate since he does not describe the protocols of the experiment or the controls used. Vogel and Minning (1953) were unable to transfer passively resistance to monkeys with *S. japonicum* antiserum. Stirewalt and Evans (1953) were unsuccessful in passive transfer of protection to mice with hyperimmune *S. mansoni* rat serum.

In our laboratory we have been conducting passive transfer experiments for several years with serum from animals infected with *S. douthitti* with negative results. We have injected various doses of homologous and heterologous hyperimmune serum for varying lengths of time prior to, and following, infection. We injected serum at the site of cercarial penetration prior to and after exposure. The results of these experiments are tabulated in Table 4. In some instances, mice receiving heavy doses of immune and normal serum were rendered more susceptible to infection than the controls.

Another approach to this problem was made by Levine (unpublished). He bathed cercariae in diluted normal and immune serum for varying lengths of time prior to exposure. These studies are still in progress but may show that there are antibodies in immune serum which may impair the ability of the cercariae to develop to maturity in the host.

Serology

The diagnosis of infection has been the principal goal of serological tests. The detection of eggs in feces or urine may be very reliable in an acute infection, but for chronic or light infections even with improved methods for concentration of eggs the intermittent escape of eggs from the intestine makes diagnosis uncertain. For this reason a greater reliance on immunodiagnostic methods has been the trend in recent years. In addition to diagnosis, serological methods have been used to evaluate the efficacy of therapy, and for studies of the serological specificity of various life cycle stages of the schistosome parasite as potential sources of antigen.

Complement Fixation

The complement fixation test has been in use in the diagnosis of schistosomiasis for close to fifty years (Taliaferro, 1929). The principal

TABLE 4

PASSIVE TRANSFER EXPERIMENTS WITH ANTISERUM OF ANIMALS INFECTED WITH *Schistomatium douthitti*

Exp. No.	Number of Mice Autopsied			Serum Dose	No. Infecting Cercariae	Worms Recovered		
	Immune Serum	Normal Serum	No. Serum			Immune Serum	Normal Serum	No Serum
				Hyperimmune Mouse Serum				
1	3	2	4	.5–1.0 ml×1	50	20.0	20.5	38.5
2	6		6	.5– ml×5	25	6.7		7.8
3	8	8	6	.25–.5 ml×6	25	8.0	11.7	10.3
4	8		8	.5 ml×5	25	8.8		8.4
5	4	4	4	.25 ml×8	50	24.0	25.0	9.0
				Hyperimmune Rabbit Serum				
6	4	2	3	3.0×1	50	—	41.5	25.6
7	7	7	5	1.5×.5	25	11.0	10.7	11.3
8	5	5	5	0.5×7	10	4.4	5.6	4.4
9	8	6	6	0.5–1.0×17	25	10.5	—	10.0
				Hyperimmune Monkey Serum				
10	6	0	6	0.25×5	25	4.0		6.6
11	0	8	7	0.25×6	25		5.7	7.0
12	5	0	5	0.25×5	25	8.4		10.2
13	5	0	5	0.3–0.8×3	25	11.0		9.2
				Serum Injected at Site of Penetration at Time of Infection				
15	3	3	3	.5 ml×1	50–150	29	15	28
				Serum Injected at Site of Penetration before Exposure				
16	3	3	3	.5 ml×1	100	21.3	5.0	31.0

problem in the use of this test has been the development of a suitable antigen. The early Japanese workers utilized alcoholic and saline extracts of adult worms (Yoshimoto, 1910). A cercarial antigen of S. *spindalis* made from infected snail livers was introduced by Fairley (1919). As reviewed by Taliaferro (1929) the early workers were concerned with the nature of the antigen, whether it was protein (LeBas, 1922), or lipoidal in character (Fairley, 1925). The tendency for schistosome antigens to react with syphilitic serum led Chaffee, *et al.*, (1954) to prepare an improved antigen from desiccated adult schistosomes extracted with cold anhydrous ether prior to extraction with buffered salt solution, which would not cross-react with non-schistosome sera. This antigen proved to be very sensitive and specific in detecting schistosomiasis in a survey of Puerto Rican soldiers (Horstman, *et al.*, 1954). Eliahim & Davies (1954), and Davies and Eliahim (1954), evaluated antigen prepared from infected snails and adult S. *mansoni* worms by a variety of methods. They found

in contrast to Mayer and Pifano (1945*a*) and Williams (1947) that adult worm antigens of *S. mansoni* were superior to cercarial antigens. They prepared a lipo-protein antigen by extracting adult worms with Coca's solution (0.5g. NaCl, 0.05g $NaHCO_5$, 0.4g phenol, distilled water to 100 ml). Schneider, *et al.*, (1956, 1957) prepared a non-dialyzable aqueous extract of adult *S. mansoni* worms that is primarily carbohydrate and nucleoprotein, and which shows promise. Lurie, *et al.*, (1952) reported that the CF test was still the most sensitive test in the diagnosis of early schistosomiasis.

Precipitins

Precipitins play a unique role in helminth immunity. They are formed in the host in response to antigenic material secreted and excreted by the parasite and in response to the somatic antigens of the dead worm. They have been implicated in effecting immobilization of helminths in the tissue of the host, stunting of growth, prevention of food assimilation and inhibition of enzymatic activity (Taliaferro, 1940, 1943).

Antigens prepared from dried livers of *Australorbis glabratus* infected with *S. mansoni,* thoroughly extracted with ether-alcohol followed by a final extraction with Coca's solution, proved to be effective in a precipitin test developed by Taliaferro, Hoffman and Cook (1928). The precipitin test has not been readily accepted in diagnosis because of cross-reaction with syphilitic serum. In contrast to the complement-fixation test, alcoholic extracts of schistosome worms are not active in the precipitin test (Miyaji and Imai, 1928).

Oliver-Gonzalez, *et al.*, (1955) utilized the precipitin test in their studies of schistosome serology. Saline extracts of adults, cercariae and eggs of *S. mansoni* were tested by "ring tests" in microprecipitation tubes with sera of rabbits injected with adults, cercariae, and eggs of *S. mansoni.* In addition, sera from acute (early) and chronic (late) infections in humans were tested. They found that in the sera of individuals infected from 40-240 days (early infection) the titer of cercarial precipitins was higher than in the sera of chronic patients. Adult antigen titers were fairly constant for both groups. Rabbits immunized with the three stages of the life cycle produced stage-specific antibodies. Precipitin tests with adult worm antigens performed by Okabe, *et al.*, (1954) were reported to be more sensitive than skin tests in the diagnosis of infection.

Circumoval Precipitin Test

Precipitins will form about living eggs incubated in sera of infected humans and monkeys when placed at 37°C for periods up to 24 hours (Oliver-Gonzalez, 1954). The precipitates which appear are antigen-anti-

body complexes formed by secretions or excretions of the living miracidium in the egg and specific antibodies in the serum. This was shown to be so by tagging immune globulin with fluorescent isocyanate and demonstrating that the precipitates around eggs incubated in immune tagged serum were fluorescent (Lewert, Lee & Jackson, unpublished). The specificity of this reaction was shown by incubating eggs in immune serum plus tagged normal serum. The precipitates in this preparation did not fluoresce. Anti-egg antibodies in immune serum could not be absorbed with adult or cercarial antigens but only with living or lyophilized eggs (Oliver-Gonzalez, *et al.*, 1955b). The circumoval precipitin test was reported to be species specific (Oliver-Gonzales, *et al.*, 1955b). When mixtures of eggs of S. *mansoni*, S. *japonicum* and S. *haematobium* are incubated together in different antisera, a precipitate will form around the homologous egg. A slight cross-reaction between the eggs of S. *mansoni* and antiserum of S. *haematobium* and of S. *japonicum* was observed.

The circumoval precipitin test was found to be more reactive in chronic schistosomiasis. From 49.5-72.8 per cent of eggs were positive for precipitates in antisera from chronic schistosomiasis cases as compared to 29.4-53.2 per cent in recent acute infections. The serum of animals exposed to worms of one sex (and in the case of female S. *mansoni* producing no eggs) was negative for circumoval antibodies. The circumoval activity is greatly diminished in sera of treated individuals (Oliver-Gonzalez, Ramos and Coker, 1955). The test therefore has diagnostic and prognostic value in evaluating therapy. Approximately 120-180 days after successful therapy the circumoval precipitin test becomes negative.

Newsome and Robinson (1956), and Robinson (1956b), reported circumoval precipitin titers in sera of baboons infected with S. *mansoni*. Robinson (1956*b*), also observed precipitates around the oral sucker of 21-day-old schistosomulae in immune serum.

Cercarienhüllen Reaction (*CHR*) of Vogel and Minning (1949a, 1949b)

Papirmeister and Bang (1948) reported the formation of a precipitate around cercariae of *Schistosoma mansoni* incubated in sera of monkeys and humans infected with schistosomiasis. This reaction, which occurred in fresh serum, was destroyed by heating the serum at 56°C for 30 minutes, and could be restored by the addition of complement. Vogel (1948), and Vogel and Minning (1949a, 1949b) published their observations on the formation of a membrane (CHR reaction) around the tail and body of living cercariae placed in immune serum. This transparent envelope which surrounds the cercariae did not kill the organism. They studied the activity of this antibody in serum from experimentally infected

mice, rabbits, dogs, monkeys, and cats and in serum from naturally infected humans. They observed that precipitates form around cercariae in fresh serum and expressed the belief that this reaction was not related to the CHR and was similar to the reaction described by Papermeister and Bang (1948). They found that the CHR reaction was negative 5-7 months after treatment and cure in infected humans. The CHR antibody appeared in the serum 40-47 days after infection in experimental animals. The formation of a membrane around schistosome cercariae was very specific and did not develop around non-schistosome cercariae.

Mayer and Pifano (1951), and Pifano and Ron Pedrique (1957) tested the serum of patients passing eggs of *S. mansoni*. They were able to detect the CHR antibody in only 79.62% and 78% of their samples, respectively. This led them to conclude that the CHR test was not as reliable as other serological methods in the diagnosis of schistosomiasis. Mayer and Pifano (1951) also reported that the CHR antibody was detected in the sera of animals infected with worms of one sex after the worms became sexually mature. Standen (1952), Meleney and Moore (1954), Stirewalt and Evans (1955), Hendricks and Cort (1956), have also reported on the CHR phenomenon. Electrophoretically-separated immune serum revealed that the CHR membrane would only develop in the gamma and alpha fraction of the serum (Evans, Stirewalt and MacKenzie, 1955). Electron micrograph studies of cercariae in immune serum indicated that the membrane is formed from a thin film of secretory substance which is present on the cuticle when the cercariae emerge from the snail (Kruidenier and Stirewalt, 1955).

In our studies of the CHR antibody we injected rabbits with saline extracts of all stages of the life cycle of *S. douthitti* and were able to observe CHR reactions in all these antisera. Sera from rabbits immunized with another trematode species (*Haematoloechus medioplexus*) were negative for CHR antibody. The CHR antibody could be absorbed with massive concentration of living cercariae. We exposed hamsters, mice, guinea pigs, rats and rabbits to infection with *S. mansoni*. The CHR was detected in our studies as early as 10 days after exposure in the rabbit, 20 days in the hamster, 34 days in the guinea pig, 40 days in the rat and 52 days in the mouse. Sera of monkeys exposed to *S. douthitti* were positive for antibody 15 days after exposure. The CHR reaction was influenced by the severity of infection. Fewer cercariae showed membranes in sera from light than from heavy infections. In one instance the CHR was negative in a monkey shown to be immune to reinfection by challenge with *S. douthitti*. Positive reactions with cercariae of *S. mansoni* and *S. douthitti* were observed in infected human sera (Kagan 1955). We also studied the appearance of CHR in animals injected with a cercarial an-

tigen (Kagan & Levine, 1956). We injected 16 different species and each host developed a CHR antibody titer. CHR titers declined after the end of cercarial immunization. There was no correlation in these hosts between the appearance of the CHR after injection of antigen or after infection with cercariae. The appearance of the CHR in the serum after the start of antigen injection varied considerably from species to species.

The CHR reaction as a diagnostic tool has limitations. The test lacks sensitivity in the diagnosis of human infections (Mayer & Pifano, 1951), may cross-react with bird schistosome cercarial antibodies (Hendricks & Cort, 1956), and necessitates having living cercariae in the laboratory. This reaction must therefore be regarded as one of the less desirable diagnostic methods available to the parasitologist.

From a biological point of view the CHR reaction holds a great deal of interest. I have called it a "modified surface precipitin phenomenon" (Kagan, 1955b). Thought of as a precipitin phenomenon between a living organism and immune serum antibodies one finds parallels with *in vitro* reactions of other helminth species in immune serum (Taliaferro, 1940). This type of precipitin phenomenon is not limited to helminths. Similar *in vitro* manifestations such as immobilization of motile organisms, accumulations of semisolid products about the periphery of individual cells, have been reported for a variety of microorganisms (Harrison, 1955).

Flocculation Tests

Shortly following the publication of a flocculation slide test for trichinosis (Sussenguth and Kline, 1944), this technique was applied in the diagnosis of schistosomiasis (*S. mansoni*) by Brandt and Finch (1946). These workers used as antigen cholesterol particles coated with an alkaline phosphate buffer extract of dried adult worms plus lecithin. The test in their hands had a high degree of sensitivity. Wright, *et al.*, (1947) used a bentonite flocculation test in the diagnosis of *S. japonicum* in military personnel. They were able to report 77.3% of infected patients with their method. Bozicevich and Hoyem (1947) working with treated individuals found the flocculation test unreliable in diagnosis.

The ease and rapidity with which the flocculation test may be performed and read makes this test a very desirable serological procedure to develop.

Agglutination

Schistosome polysaccharide, and other helminth polysaccharide fractions, is able to inhibit the alpha and beta agglutinins of human serums and the Forssman hemolysin of rabbit serum (Oliver-Gonzalez and Torregrosa, 1944). Adult schistosome worms incubated in human serum reduce

the A_2 isoagglutinin titer to zero (Oliver-Gonzalez and Gonzalez, L. M., 1949). Sheep cell agglutinins present in 81.7% of 120 normal human sera are reduced to zero after incubation with living *S. mansoni* worms. The relationship between sheep red cell agglutinogens, the A_2 human blood substance and parasite antigens is not well understood at present. It is thought that perhaps the organism secretes or excretes an A_2 isoagglutinogen-like substance which is antigenic and which absorbs these agglutinins from the serum during active infection (Oliver-Gonzalez, 1952).

We attempted to determine whether Forssman antigen was present in schistosome cercariae. We exposed 3 rabbits which had developed high Forssman titers 6 months earlier as a result of immunization with sheep red cells. After exposure to massive doses of cercariae, complement fixation tests were made with the sera of these rabbits to determine if there was any rise in the Forssman titer (anamnistic effect). These rabbits showed no rise in titer and we concluded that under the conditions of the test no Forssman antigen could be detected.

Cercarial agglutinins were reported in the sera of individuals and animals infected with *S. mansoni* by Liu & Bang (1950). These workers found that living cercariae were agglutinated in the sera from acute human infections and the sera from 4 to 7 chronic infections showed reduced activity.

Standen (1952) reported that normal cattle sera agglutinated cercariae. Stirewalt and Evans (1955) found that agglutination of cercariae was related to the CHR reaction.

Oliver-Gonzalez, *et al.*, (1955a) reported cercarcial agglutinin titers in immunized rabbits injected with lyophylized cercariae and in sera of adult human infections. Following the pattern in the precipitin test, cercarial agglutinin titers of early schistosome cases were higher than in chronic late cases. In infections in mice with worms of one sex, where the circumoval precipitin titer was zero, cercarial agglutinin titers as high as 1:32 were observed.

In our laboratory we studied cercarial agglutination in the sera of normal and immunized animals (Kagan & Levine, 1956). The normal cercaricidal activity of serum masked the agglutinin antibody in all but cow and horse sera. When the cercaricidal factor was destroyed by heat, normal sera from 8 additional species agglutinated cercariae. The sera of 16 animal species agglutinated cercariae after being injected with a cercarial antigen. The number of cercariae that were agglutinated varied from 10-100% in some sera. Agglutinin titers of immune serum varied from 1:32 for the rabbit to 1:256 for the cow. The presence of CHR antibody inhibited the agglutination of cercariae. In sera in which the titer of the CHR antibody was exceeded, agglutination of the cercariae took place.

Hemagglutination

The presence of agglutinins in serum of antigen-injected animals led us to look for a more sensitive method of measuring these antibodies. We therefore investigated the use of tannic acid hemagglutination of sensitized sheep red cells.

The hemagglutination test used was the tannic acid modification introduced by Boyden (1951). Sheep red cells were sensitized with tannic acid and coated with a cercarial antigen (Kagan, 1955*a*). One drop of these coated cells was added to a series of dilutions in 0.5 ml. amounts of antiserum. The test was read from the pattern formed by the cells on the bottom of the tube. In a negative serum, the cells fall to the bottom and form a doughnut-shaped bullseye. In a positive serum, the cells agglutinate and form a thin layer of cells over the bottom of the tube.

One of the first findings in our hemagglutination studies was the differentiation of agglutinins found in normal serum and in serum of infected animals. Normal horse and cow serum (which actively agglutinates cercariae) is negative by hemagglutination. Agglutinins found after immunization will agglutinate sensitized sheep cells (Kagan, 1955*a*).

In our initial study we were also able to demonstrate that agglutinins were formed in rabbits in response to the injection of every stage of the schistosome life cycle. The test was specific for schistosomiasis, since antisera made against frog lung flukes (*Haematoloechus medioplexus*) were negative by hemagglutination.

The hemagglutination test was more sensitive than the CHR test, more sensitive than cercarial agglutination test but less sensitive than a miracidial immobilization test (Senterfit, 1953) in detection of antibody in immune serum.

During the summer of 1955 I was privileged to work in the laboratory of Dr. Oliver-Gonzalez. We studied the hemagglutination test with a variety of antigens. Cells were coated with antigens from cercariae, adults and eggs of *S. mansoni.* In addition, egg antigens of *S. japonicum, S. haematobium* and *Schistosomatium douthitti* were used and a cercarial and adult metabolic product antigen of *S. mansoni.* These antigens were tested against sera of rabbits injected with eggs, cercariae and adults of *S. mansoni,* sera from acute cases of infection in children with *S. mansoni,* and sera of patients before and after treatment. In contrast to the precipitin test, we were not able to demonstrate any marked stage specificity with this method. In 4 of the 5 children's sera tested, the egg antigen was the most sensitive antigen. With egg antigen and adult metabolic product antigen we were able to demonstrate differences in the titer of 4 of 10 sera from patients before and after treatment (Kagan and Oliver-Gonzalez, 1956).

Miracidial Immobilization Test

Senterfit (1953) published on the immobilization of miracidia in immune serum. He studied the reaction in serum of infected hamsters and monkeys. In the sera of infected monkeys, immobilization antibodies appeared 39-48 days after infection with *S. mansoni* and rose to a titer of 1:160 by the 75th day. In general the miracidial immobilization titer paralleled the cercarial agglutination titer for these animals. Monkeys infected with *S. japonicum* for 5½ years had a titer of 1:10.

We observed and studied the miracidial immobilization phenomenon independently of Senterfit's work (Kagan, 1955*b*), and found this test to be very sensitive in detecting antibodies in serum of antigen-injected and infected animals. All stages of the schistosome life cycle when injected into rabbits produced immobilization antibodies. Immobilization titers were observed in sera of monkeys and humans infected with *S. mansoni.* Immobilization titers were stronger with homologous miracidia. In certain immune sera, miracidia were agglutinated at the posterior end of the organism into "rosettes."

Miracidial agglutinins, circumoval precipitins, cercarial agglutinins, and complement fixation antibodies were studied by Lewert and Lee (unpublished). They fractionated a human serum from infection with *S. mansoni* on a starch block (Kunkel, 1954) and recovered 50 fractions. They observed the maximum immunological reaction in the following fractions: Circumoval precipitates, fraction 38 (range 32-42); miracidial agglutination, fractions 38-40 (range 32-50); cercarial agglutination, fractions 40-42 (range, 36-46); complement fixation adult antigen, fractions 42-44 (range 32-50), complement-fixing cercarial antigen, fractions 44-46 (range 32-50). Fractions 35-46 represent the gamma globulin portion of the immune serum. The miracidial agglutinins were by far the most sensitive antibody.

This ionographic approach to the serology of helminthiasis is a most useful tool. Perhaps with this method more sensitive and specific diagnostic antigens will be developed by fractionating complex helminth whole worm or tissue antigens.

Skin Tests

Because of its relative simplicity, ease of application and rapid result, the skin test as a diagnostic epidemiological tool has appealed to workers in endemic areas. A perusal of the literature reveals that the test has had to overcome lack of specificity and standardization of what constitutes a positive test. Pellegrino (unpublished) has reviewed "The Intradermal Test in the Diagnosis of Schistosomiasis" for the African Conference on Schistosomiasis, in Brazzalville, Belgian Congo (1956).

Practically every stage of the life cycle of the schistosome parasite has

been used for antigen: Adult worms (Mayer and Pifano, 1949, Coutinho, 1949, 1952), cercariae (Oliver-Gonzalez and Pratt, 1944, Pratt and Oliver-Gonzalez, 1947), infected snail livers (Fairley and Williams, 1927), miracidia (Sherif, 1956) and eggs (Oliver-Gonzalez, 1954). Various workers have claimed greater sensitivity with alcoholic extracts, saline extracts, polysaccharide fractions (Martins, 1949) or delipidized powders.

According to Pellegrino (unpublished) the following areas have to be developed: (1) Standardization of the cutaneous test on an objective basis, (2) availability of sufficient quantities of antigen for the intradermal test in epidemiological surveys, (3) use of purified antigens, (4) biological standardization of antigens, (5) evaluation of the skin test as a criterion for chemotherapeutic cure. I agree with him fully.

With regard to point (1) it is important for workers in any aspect of immunology and serology to quantitate their antigenic material in terms of nitrogen, protein or other chemical entities. Without chemical quantitation a 1:1000 dilution of an antigen in laboratory A could be equal to a 1:100 dilution in laboratory B. With regard to obtaining large amounts of antigen, there are published techniques for obtaining cercariae in quantity (Standen, 1950, Pellegrino and Macedo, 1955), miracidia (Sherif, 1956), adult worms (Yolles, *et al.*, 1949) and eggs (Coker and Lichtenberg, 1956).

Attempts have been and are being made to purify schistosome antigens. Research in this area must continue and with new techniques available to parasitologists such as serum-agar methods for assay of antigens (Kagan, 1957), ionographic methods for physical separation of antigens, chemical methods for isolation of specific fractions (Schneider, *et al.*, 1956), purified antigens can be made available to the clinician or public health worker in the near future.

Pellegrino, Memoria and Macedo (1957) recently demonstrated that a skin-testing antigen can be biologically assayed and objective criteria for a positive skin test established. They were able to show that when 0.01-0.05 ml of antigen was injected subcutaneously there was a linear relationship between the concentration of the antigen and the mean area of the wheal.

Several reports have appeared regarding the evaluation of chemotherapy and cure by means of the skin test. Oliver-Gonzalez and Pratt (1944) evaluated a cercarial antigen and were able to show that skin sensitization persisted years after cure. Oliver-Gonzalez, Bauman and Bennenson (1954, 1955*a*) reported that in 104 Puerto Rican individuals infected with S. *mansoni*, 35.6% were positive with an egg antigen of S. *mansoni*, whereas 95.2% were positive with a cercarial antigen. Skin tests on patients passing viable eggs were negative with an egg antigen,

whereas they were positive in those patients treated a year earlier. These results were elaborated in further studies in conjunction with the circumoval precipitin test. The negative skin tests in a treated patient became positive approximately 183 days after treatment. As a result of the treatment, the circumoval precipitin test becomes negative. A negative circumoval test and a positive skin test following treatment appears to be indicative of the termination of infection (Oliver-Gonzalez, Romas and Coker, 1955). Sherif (1956), using a miracidial antigen, reported that the skin test became negative in patients 3-6 months after cure.

The presence of metabolic antigens in the egg antigen probably accounts for the differences between the response of the miracidium and the egg antigen in the skin test. Sherif utilized the hatched and actively swimming miracidium as his source of antigen and did not observe a negative skin test during active infection. The shell of the egg in the egg antigen is probably non-specific and may mask or block the antigenic stimulation of the miracidium in the enclosed shell, which may explain why the miracidium in the egg antigen does not cause a positive test to develop. The fluid within the egg, however, can escape as evidenced by the formation of precipitates in the circumoval precipitin test, and acts as the antigenic stimulus in the skin test. The fluid bathing the miracidium is probably rich in secretory and excretory miracidial metabolic products. During active infection there may be an excess of antigen in the serum of the host which absorbs and binds the antibody available to the egg antigen. The skin test is therefore negative in the patient with an active infection. This phenomenon (negative test in the face of active infection) was also reported by Lurie, *et al.*, (1953) in skin tests with cercariae with children infected with *S. haematobium*. Approximately 6 months after cure the skin test with the egg antigen becomes positive. As a clinical criterion for cure this interval of time is too long. Perhaps if greater concentrations of metabolic antigens from adult and larval worms could be utilized in the skin test a shorter time interval between death of the worms and a positive skin test would result. The sero-evaluation of chemotherapeutic cure of helminth disease offers a challenging area in the serology of parasitic disease.

Prausnitz-Küstner Reaction

The P-K test (Prausnitz-Küstner) is based on the passive transfer of sensitivity to the skin of a normal individual by subcutaneous injection of serum containing antibody. Twenty-four hours later the site is injected with antigen and a wheal and erythema indicates a positive test. This is the standard test for atopic or reagin antibody of allergies. Taliaferro and Taliaferro (1931) were the first to demonstrate this reaction in schisto-

somiasis with sera of infected individuals. Pellegrino (unpublished) was able to demonstrate the P-K phenomenon in his patient's serum. Guerra, Mayer and Di Prisco (1945) demonstrated that the reaction was specific for schistosomiasis, since *Fasciola hepatica* antigen did not elicit a positive response on passive transfer.

Ritchken and Gelfand (1954) and Ritchken (1955) called attention to the allergic condition associated with infection 4 to 10 weeks after exposure to schistosomiasis. They believe that "River-Fever" or "Katayma Syndrome" associated with urticaria, fever and eosinophilia are the allergic manifestations of schistosomiasis.

Attempts to neutralize the antibody in the serum by absorption with antigen prior to injection into the skin of a donor were unsuccessful (Taliaferro and Taliaferro, 1931; Pellegrino, unpublished). These tests indicate that perhaps a true atopic or univalent antibody was being demonstrated in schistosomiasis. Coker and Oliver-Gonzalez (1956) were able to passively transfer immune serum to the skin of normal individuals and observe a positive skin test at the site of injection 24 hours later with egg antigen. In the skin of infected individuals the passive transfer was negative.

It is not clear from the preliminary report by Coker and Oliver-Gonzalez (1956) whether the serum was absorbed prior to passive transfer into the skin of the normal or infected individual. If a true atopic antibody is neutralized in the infected individual this reaction should be investigated further to determine how soon after cure the negative test becomes positive. This reaction may prove to be more sensitive than the immediate skin test in the evaluation of the curative effects of therapy.

Discussion

The host-parasite relationship of *Schistosomatium douthitti* (and other schistosome species) is complex and the possibility that more than one immunological mechanism may be operative in a single host or in several different hosts should not be ruled out. In the monkey infected with *S. douthitti,* the death of the worms in massive infection may indicate an antigen-antibody response or perhaps a metabolic or enzymatic impasse. In the rat the infection is eliminated by what appears to be an active cellular response. Perhaps there is an antigen-antibody basis for this resistance, or perhaps a non-specific response such as an increase in properdin levels in the serum of the host. In the mouse, a light infection will persist for the life time of the host, and whether the limited protection that an initial infection renders the host is dependent on an antigen-antibody basis or on an enhancement of non-antibody mechanisms remains to be experimentally demonstrated. Thompson (1954) believed that ac-

quired immunity against *Schistosoma mansoni* in experimental hosts was mediated through an enhancement of innate immunity. Stirewalt (1953) demonstrated the penetration of *S. mansoni* cercariae in mice within 24 hours after initial exposure was impeded by a non-specific mechanism in the host. Lindquist (1950) reported that the larvae of *Nippostrongylus muris*, the species with which Taliaferro and Sarles conclusively demonstrated an antigen-antibody basis for immunity in the rat (Taliaferro, 1943), is encapsulated and killed in the skin of cotton rats in the apparent absence of antibodies.

I personally believe that the immunology of schistosomiasis has an antigen-antibody basis. However, until this point is unequivocally proven by passive transfer experiments I believe that one should not rule out other kinds of immunological mechanisms that are not based on antibodies. The immunity to *Bacillus anthracis*, the causative agent of anthrax, is pertinent in this regard. In the mouse, acquired immunity to anthrax is dependent on the stimulation by capsular antigens and follows classical antigen-antibody priciples established for pneumococcus and other organisms. In the sheep, rabbit and guinea pig, in spite of the fact that acquired immunity can be so dramatically demonstrated with heat-attenuated organisms (which is fortunate since Pasteur used this organism in his dramatic demonstration of acquired immunity in sheep at Pouilly-le-Fort in 1881), the mechanism of this immunity is still not clearly elucidated but appears to be independent of capsular antigens and may be of a non-antibody type (Raffel, 1953).

The different response of hosts infected with schistosomes may have important bearing on our understanding of the immunology of schistosomiasis in man. From preliminary reports, monkeys infected with varying numbers of *S. mansoni* cercariae appear to respond differently after exposure (Naimark, *et al.*, 1957*a*). Heavily infected animals become refractive to reinfection whereas lightly infected animals do not. In areas such as Puerto Rico, where the incidence of schistosomiasis is about 10% (White, *et al.*, 1957) and from an epidemiological point of view is an area of light infection, the infection appears to be of long duration in man. This means that unless the infected individuals who come to the United States from Puerto Rico are treated, they may remain infected for life. From a public health point of view this is an important consideration and studies should be initiated in our larger cities where there are concentrations of Puerto Rican citizens to resolve this point.

From the information derived from animal experimentation, I believe we can make some hypotheses concerning the effect of treatment on immunity and resistance in man. Schwetz (1956) believed that for humans infected with schistosomes, a state of "premunition" exists. This type of

immunity necessitates the presence of an active infection to maintain and stimulate immunity. If this be true, then a clinical cure by chemotherapy would in time deprive the individual of his immunity. Schwetz therefore infers that treatment should be initiated to alleviate the patient's chronic symptoms but not to effect a complete cure. Our studies on the effect of chemotherapy on duration of immunity in mice (Kagan and Lee, 1952) supports the above hypothesis; however, studies in monkeys (Vogel and Minning, 1953, Kagan, 1953, Meleney and Moore, 1954) have shown that animals cured by chemotherapy or by "self-cure" are immune to reinfection years after the termination of the initial infection. I suspect that the immune response in man is similar to the mechanism of immunity in the monkey and differs from the immune response in the mouse. Therefore a clinical cure in man may not strip him of any acquired immunity against reinfection with schistosomes.

The immunodiagnosis of schistosomiasis is at a very formative stage. Many serological tests have been developed and the antigenicity of practically every stage in the life cycle of the parasite has been probed. Serological tests still fall short in detection of early infection (Naimark, *et al.*, 1957*b*) and early chemotherapeutic cure. Perhaps we will find the answers in refined techniques and extraction of more potent somatic antigens from the various life cycle stages of the parasite. This avenue of research must be pursued; but, I believe that much more emphasis must be directed toward collecting metabolic antigens and evaluating their efficacy in the immunology and serology of schistosomiasis.

As Newsome (1956) outlined in his recent review, there are many questions to be resolved in the immunology of schistosomiasis. With the application of modern research methods and ideas these problems will be solved in the near future.

Summary

Contributions made by the author and his co-workers on the immunology and serology of *Schistosomatium douthitti* and *Schistosoma mansoni*, have been discussed. In addition, the contributions of other workers have been reviewed. The discussion of immunology has been divided into innate immunity, natural acquired immunity, artificially acquired immunity, natural passive transfer of immunity and artificial transfer of immunity. The discussion on the serology has been subdivided into the following divisions: complement-fixation test, precipitins, circumoval precipitin test, CHR (cercarianhüllen reaction of Vogel and Minning), flocculation tests, agglutination, hemagglutination, miracidial immobilization test, skin tests and Prausnitz-Küstner reaction.

ACKNOWLEGEMENTS

The investigations of the author and his co-workers on schistosomiasis were made possible in part by a National Research Council Postdoctoral Fellowship sponsored by Dr. William H. Taliaferro, Chairman, Department of Microbiology, University of Chicago, during the years 1950-1952. I wish to thank Dr. Taliaferro for his encouragement and sustained interest in my research. From 1952-1957, when the author was at the University of Pennsylvania, research was supported by a grant from the National Institutes of Health, Division of Allergy and Infectious Diseases, Public Health Service.

REFERENCES

Berberian, D. A., Paquin, H. O., Jr., Fantauzzi, A. 1953. Longevity of *Schistosoma haematobium* and *Schistosoma mansoni:* Observations based on a case. *J. Parasitol.* **39,** 517-519.

Bouillon, A. 1950. Bibliographie des schistosomes et des schistosomiasis (Bilharzioses) humaines et animales de 1931 a 1949. *Mem. Inst. Royal Colonial Belge* **27,** 1-141.

Boyd, W. C. 1956. *Fundamentals of Immunology.* 3rd ed. 776 pp. Interscience Pub.

Boyden, S. V. 1951. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. *J. Exp. Med.* **93,** 107-120.

Bozicevich, J., & Hoyem, H. M. 1947. Intradermal and serological tests in patients with schistosomiasis japonica. *Nat. Inst. Health Bull.* **189,** 199-212.

Brandt, J. L., & Finch, E. P. 1946. A simple flocculation slide test for the diagnosis of schistosomiasis. *Am. J. Clin. Path.* **16,** 141-152.

Büttner, A. 1953. Sur l'aptitude de certains rongeurs sauvages a devenir reservoirs de virus de la bilharziose intestinale humaine (Note preliminaire). *Bull. Soc. Path. Exot.* **46,** 688-692.

Cardoso, W. 1953. A esquitosomiase mansonica no negro. *Medicina, Cirurgia Farmacia,* Rio de Janeiro, **202,** 89-95.

Chaffee, E. F., Bauman, P. M., and Shapila, J. J. 1954. Diagnosis of schistosomiasis by complement-fixation. *Am. J. Trop. Med. & Hyg.* **3,** 905-913.

Chandler, A. C. 1953. The relation of nutrition to parasitism. *J. Egyptian Med. Assoc.* **36,** 533-552.

Cohn, M. 1953. Production of antibodies in experimental animals. *Methods in Med. Res.* **5,** 271-283.

Coker, C. M. 1957. Effect of cortisone on natural immunity to *Schistosoma mansoni* in mice. *Proc. Soc. Exp. Biol. & Med.* **96,** 1-3.

Coker, C. M., & Lichtenberg, F. 1956. A revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. *Proc. Soc. Exp. Biol. & Med.* **92,** 780-782.

Coker, C. M., & Oliver-Gonzalez, J. 1956. Studies on immunity to schistosomiasis—passive transfer of anti-egg antibody in humans. *Am. J. Trop. Med. & Hyg.* **5,** 385.

Coutinho, J. O. 1949. Diagnostico da esquistossomose pela intradermoreacao com antigenos de esquistosomas adultos. *Rev. Clin. Sao Paulo* **25,** 7-15.

Coutinho, J. O. 1952. Nova tecnica de preparo de antigeno de vermes adultos para a intrademo—reacao na esquistossomose. *Folia Clinica et Biologica* **18,** 121-124.

Cram, E. B., and Files, V. S. 1947. Experimental mammalian infection with the schistosomes of man. 1. Laboratory animals as a source of supply of adult schistosomes and their ova. *Nat. Inst. Health Bull.* **189,** 101-105.

Culbertson, J. T. 1936. The cercaricidal action of normal serums. *J. Parasitol.* **22,** 111-125.

Culbertson, J. T., and Talbot, S. B. 1935. A new antagonistic property of normal serums: the cercaricidal action. *Science* **82**, 525-526.

Davies, A. M., and Eliakim, M. 1954. The value of different antigens in the diagnosis of chronic bilharziasis by the skin and complement fixation tests. *Am. J. Trop. Med. & Hyg.* **3**, 728-741.

Eliakim, M., & Davies, A. M. 1954. The complement-fixation test in bilharziasis. 1. The value of different extracts of *Schistosoma mansoni* and *Fasciola hepatica* worms as antigens. *Parasitology* **44**, 407-413.

Evans, A. S., Stirewalt, M. A., & MacKenzie, M. 1955. Serological reactions in *Schistosoma mansoni* infections II. Cercarial behavior in electrophoretically separated fractions of sera of infected and uninfected mice. *Exp. Parasitol.* **4**, 419-426.

Fairley, N. H. 1919. The discovery of a specific complement fixation test for bilharziasis and its practical application to clinical medicine. *J. Roy. Army Med. Corps* **32**, 449-460.

Fairley, N. H. 1925. Complement-fixation with bilharzia. Part I. The lipoidal nature of cercarial antigen as used in the complement-fixation test for mammalian bilharziasis. *J. Pathol. & Bacteriol.* **28**, 591-607.

Fairley, N. H. 1926. Studies in the chemotherapy and immunity reaction of schistosomiasis (*Schistosoma spindalis* and *S. haematobium*). *Trans. Roy. Soc. Trop. Med.* **20**, 236-267.

Fairley, N. H. 1927. The early spontaneous cure of bilharziosis (*S. spindalis*) in monkeys (*Macacus sinicus*) and its bearing on species immunity. *Indian J. Med. Res.* **14**, 685-700.

Fairley, N. H. 1951. Schistosomiasis and some of its problems. *Trans. Roy. Soc. Trop. med. & Hyg.* **45**, 279-306.

Fairley, N. H., & Williams, F. E. 1927. A preliminary report on an intradermal reaction on schistosomiasis. *Med. J. Australia* **2**, 811-818.

Fairley, N. H., Mackie, F. P., and Jasudasan, F. 1930. Further observations on spontaneous course in *Macacus sinicus*. *Ind. Med. Res. Mem.* **17**, 53-59.

Ferguson, F. E., Goodwin, M. H., Jr., Gerhardt, C. E., Klock, J. W., and Williams, E. R. Research on *Schistosomiasis mansoni* at the Puerto Rico field station of the United States Public Health Service. *Proc. Carib. San Eng. Conf.* (Nov. 1955), *Pan. Am. San. Bur.*, published by Puerto Rican Comm. Dept. Health, San Juan. In press.

Fisher, A. C. 1934. A study of the schistosomiasis of the Stanleyville district of the Belgian Congo. *Trans. Roy. Soc. Trop. Med. & Hyg.* **28**, 277-306.

Gelfand, M. 1950. *Schistosomiasis in South Central Africa—A clinical-pathological study.* 239 pp. Post-Graduate Press, Juta & Co. Ltd.

Girges, R. 1934. *Schistosomiasis (Bilharziasis).* John Bale, Sons and Danielsson, Ltd. London. 527 pp.

Gönnert, R. 1955. Schistosomiasis-Studien. II. Über die Eibildung bei *Schistosoma mansoni* und das Schichsal der Eier im Wirtsorganizmus. *Ztschr. Tropenmed. u. Parasitol.* **6**, 33-52.

Gönnert, R., and Vogel, H. 1955. Über die Abhängigkeit des Therapieerfolges von Wirts—und Parasitenstamm bei der experimentellen Schistosomiasis. *Ztschr. Tropenmed. u. Parasitol.* **6**, 193-198.

Guerra, P., Mayer, M., & Di Prisco, J. 1945. La especificidad de la intradermorreacciones con antigenos de *Schistosoma mansoni* y *Fasciola hepatica* por el metado de Prausnitz-Kuestner. *Rev. Sanid. y Asist. Soc.* **10**, 51-63.

Harrison, J. A. 1955. General aspects of immunological reactions with bacteria and protozoa. In *Biological Specificity and Growth*, Chap. 7, 141-156. Princeton Univ. Press.

Hendricks, J. R., & Cort, W. W. 1956. A study of the *in vitro* actions of antiserums on the cercariae of certain bird schistosomes. *J. Parasitol.* **42**, 557-564.

Horstman, H. A., Jr., Chaffee, E. F., and Bauman, P. M. 1954. Schistosomiasis mansoni in Puerto Rican soldiers. *Am. J. Trop. Med. & Hyg.* **3**, 914-917.

Hsü, H. F., and Li, S. Y. [in press]. Studies on *Schistosomiasis japonica* in Formosa IV. Wild rats and other small mammals as reservoir hosts of *Schistosoma japonicum* in Formosa. *Proc. 8th Pacific Sci. Congr.* Quezon City, Nov. 1953. In press.

Hunter, G. W., III, Ritchie, L. S., Lin, S., Pan, C., and Tanake, H. 1956. Immunological Studies. I. Experiments with bird and human schistosomes in small mammals. *Exp. Parasitol.* **5**, 551-559.

Iralu, V. Personal communication.

Kagan, I. G. 1952. Acquired immunity in mice infected with *Schistosomatium douthitti. J. Inf. Dis.* **91**, 147-158.

Kagan, I. G. 1953. Experimental infections of rhesus monkeys with *Schistosomatium douthitti* (Cort, 1914). *J. Inf. Dis.* **93**, 200-206.

Kagan, I. G. 1955*a*. Hemagglutination after immunization with schistosome antigens. *Science* **122**(3165), 376-377.

Kagan, I. G. 1955*b*. Studies on the serology of schistosomiasis. I. The *in vitro* activity of cercariae and miracidia in serum of experimental natural and immunized hosts. *Exp. Parasitol.* **4**, 361-376.

Kagan, I. G. 1957. Serum-agar double diffusion studies with ascaris antigens. *J. Inf. Dis.* **101**, 11-19.

Kagan, I. G., and Lee, C. L. 1952. Chemotherapy of experimental infections of mice with *Schistosomatium douthitti, Schistosoma mansoni* and *Schistosoma japonicum. J. Inf. Dis.* **91**, 224-230.

Kagan, I. G., and Lee, C. L. 1953. Duration of acquired immunity of *Schistosomatium douthitti* infections in mice following treatment. *J. Inf. Dis.* **92**, 52-57.

Kagan, I. G., & Levine, D. M. 1956. Studies on the serology of schistosomiasis. II. The *in vitro* activity of cercariae of *Schistosoma mansoni* in sera of normal and antigen-injected animals. *Exp. Parasitol.* **5**, 48-58.

Kagan, I. G., and Meranze, D. R. 1957. The histopathology of the liver in mice experimentally infected with *Schistosomatium douthitti. J. Inf. Dis.* **100**, 32-39.

Kagan, I. G., & Oliver-Gonzalez, J. 1955. Preliminary studies on hemagglutination in schistosomiasis. *J. Parasitol.* **41**(Suppl), 26.

Kagan, I. G., Short, R. B., & Nez, M. M. 1954. Maintenance of *Schistosomatium douthitti* (Cort, 1914) in the laboratory (Trematoda: Schistosomatidae). *J. Parasitol.* **40**, 1-16.

Kawamura, R. 1932. The recent researches on schistosomiasis in Japan. *Compt Rend. Cong. Internat. Med. Trop. & Hyg.* (Cairo, Dec. 1928). **4**, 311-319.

Khalil, M. B. 1931. The bibliography of schistosomiasis (bilharziasis), zoological, clinical and prophylactic. *Publ. Fac. Med.* Egyptian Univ. 506 pp.

Kruidenier, F. V., and Stirewalt, M. A. 1955. The structure and source of the pericercarial envelope (CHR) of *Schistosoma mansoni. J. Parasitol.* **41**(Suppl.), 22-23.

Kunkel, H. G. 1954. *Methods of biochemical analysis.* **1**, 141-170. Interscience Pub. Inc.

Kuntz, R. E. 1955. Biology of the schistosome complexes. *Am. J. Trop. Med. & Hyg.* **4**, 383-413.

Kuntz, R. E., & Malakatis, G. M., and Wells, W. H. 1955. Susceptibility studies in schistosomiasis. II. Susceptibility of wild mammals to infection by *Schistosoma mansoni* in Egypt with emphasis on rodents. *Am. J. Trop Med. & Hyg.* **4**, 75-89.

LaRue, G. R. 1957. The classification of digenetic Trematoda: A review and a new system, Exp. *Parasitol.* **6**, 306-344.

LeBas, G. Z. L. 1922. On the nature of the antigen in the complement fixation test for bilharziasis. *J. Trop. Med. & Hyg.* **25**, 49-55.

LeGrange, E. 1955. Considerations sur la pathologie de la bilharziose a *Schistosoma mansoni. Ann. Soc. Belge de Med. Trop.* **6**, 719-724.

Levine, D. M. Personal communication.

Lewert, R. M., and Lee, C. L. Distribution of various reactants in human anti-*Schistosoma mansoni* serum fractionated by starch electrophoresis. Manuscript in preparation.

Lewert, R. M., Lee, C. L., & Jackson, G. Unpublished.
Lin, S., Ritchie, L. S., & Hunter, G. W., III. 1954. Acquired immunologic resistance against *Schistosoma japonicum*. *J. Parasitol.* 40(Suppl), 42.
Lindquist, W. D. 1950. Some abnormal host relationships of a rat nematode, *Nippostrongylus muris*. *Am. J. Hyg.* **52**, 22-41.
Liu, C., and Bang, F. B. 1950. Agglutination of cercariae of *Schistosoma mansoni* by immune sera. *Proc. Soc. Exp. Biol. & Med.* **74**, 68-72.
Lurie, H. I., deMeillon, B., Staffberg, N. 1952. Experimental bilharziasis in animals. I. Early diagnosis of bilharziasis. *S. Afr. Med. J.* **26**(56), 1005-1008.
Lurie, H. I., deMeillon, B., Staffberg, N., and Eiselen, H. H. 1953. Negative cercarial antigen skin tests in proven urinary bilharziasis in children. *S. Afr. Med. J.* **27**, 295-296.
Lurie, H. I., and deMeillon, B. 1957. Experimental bilharziasis in laboratory animals. V. Immunity in mice produced by repeated small infections. *S. Afr. Med. J.* **31**(4), 68-69.
Magath, T. B., and Mathieson, D. R. 1945. Infection of wild rats in Leyte with *Schistosoma japonicum*. *U. S. Naval Med. Bull.* **45**, 1195-1202.
Martins, A. V. 1949. Diagnostico de laboratorio da *Esquistosomose mansoni*. Thesis. Fac. Med. Univ. Minas. Geräis. 265 pp.
Martins, A. V., Martins, G., & de Brito, R. S. 1955. Reservatorios silvestre do *Schistosoma mansoni* no etado de Minas Geräis. *Rev. Brasileira Malariologia* **7**, 259-265.
Mayer, M., and Pifano, F. 1945. La reaccion de desviacion del complemento, segun Fairley, en la *Schistosomiasis mansoni;* estudio fundamentado en 1932 reacciones. *Rev. Sanid. y Asist. Soc.* **10**(1), 65-93 .
Mayer, M. y Pifano, F. 1949. Experiencias de ocho enos en la elaboracion y aplicacion de un antigeno de vermes adultos de *Schistosoma mansoni* para la intradermoreaccion diagnostica de la bilharziosis. *Arch. Venezolanos de Pathol. Trop. & Paras. Med.* **1**(2), 1-35.
Mayer, M., and Pifano, F. 1951. La cercaria-reaccion de Vogel-Minning en la *Schistosomiasis mansoni*. *Rev. Sanid. y Asist. Soc.* **16**, 461-474.
Meleney, H. E., und Moore, D. V. 1954. Observations on immunity to superinfection with *Schistosoma mansoni* and S. *haematobium* in monkeys. *Exp. Parasitol.* **3**, 128-139.
Miyaji, S., and Imai, B. 1928. Serologischen Studien bei *Schistosomiasis japonica*. *Centralbl. f. Bacteriol.* **106**, 237-246.
Moore, D. V., Yolles, T. K., and Meleney, H. E. 1949. A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni*. *J. Parasitol.* **35**, 156-170.
Naimark, D. H., Benenson, A. S., Oliver-Gonzalez, J., and Ritchie, L. 1957*a*. Studies on *Schistosomiasis mansoni* in primates. Observations on acquired resistance. Paper before Am. Soc. Trop. Med. & Hyg. Philadelphia, Oct. 1957.
Naimark, D. H., Oliver-Gonzalez, J., Chaffee, E. F., and Anderson, R. I. 1957*b*. Studies on *Schistosomiasis mansoni* in primates (1) Initial occurrence of serological antibodies correlated with egg recovery. *J. Parasitol.* **43**(Suppl), 26.
Newsome, J. 1956. Problems of fluke immunity with special reference to schistosomiasis. *Trans. Roy. Soc. Trop. Med. & Hyg.* **50**, 258-274.
Newsome, J., and Robinson, D. L. H. 1954. Investigation of methods of maintaining *Schistosoma mansoni* in vitro. *Ann. Trop. Med. & Parasitol.* **48**, 194-200.
Newsome, J. and Robinson, D. L. H. 1956. Reactions of *Schistosoma mansoni* eggs and schistosomulae to immune serum. *Trans. Roy. Soc. Trop. Med. & Hyg.* (Demonstration) **50**, 310-11.
Okabe, K., Koga, Y., Shibue, H., and Matsuse, M. 1954. Immunological studies on schistosomiasis japonica. 3. Intradermal and precipitin tests for *Schistosoma japonica*. *Kurume Med. J.* **1**, 85-89.
Olivier, L. 1953. Observations on the migration of avian schistosomes in mammals previously unexposed to cercariae. *J. Parasit.* **39**, 237-243.

Olivier, L. and M. Schneidermann. 1953. Acquired resistance to *Schistosoma mansoni* infection in laboratory animals. *Am. J. Trop. Med. & Hyg.* **2**, 298-306.

Oliver-Gonzalez, J. 1952. Agglutinins for sheep cells in human serums and their relationship to the A_2 isoagglutinogen-like substance in infectious organism. *J. Inf. Dis.* **90**, 44-47.

Oliver-Gonzalez, J. 1954. Anti-egg percipitins in the serum of humans infected with *Schistosoma mansoni*. *J. Inf. Dis.* **95**, 86-91.

Oliver-Gonzalez, J., and Torregrosa, M. V. 1944. A substance in animal parasites related to the human isoagglutinogens. *J. Inf. Dis.* **74**, 173-177.

Oliver-Gonzalez, J., and Gonzalez, L. M. 1949. Release of the A_2 isoagglutinogen-like substance of infectious organisms into human blood serum. *J. Inf. Dis.* **85**, 66-71.

Oliver-Gonzalez, J., Bauman, P. M., and Benenson, A. S. 1954. Intradermal response to egg antigen in humans with active and treated (Fuadin) *Schistosoma mansoni* infections. *Proc. Soc. Exp. Biol. & Med.* **87**, 186-188.

Oliver-Gonzalez, J., Bauman, P. M., and Benenson, A. S. 1955*a*. Immunological aspects of infections with *Schistosoma mansoni*. *Am. J. Trop. Med. & Hyg.* **4**, 443-452.

Oliver-Gonzalez, J., Bauman, P. M., and Benenson, A. S. 1955*b*. Species specificity of the anti-egg precipitin in schistosome serums. *J. Inf. Dis.* **96**, 95-100.

Oliver-Gonzalez, J., Ramos, F. L., and Coker, C. M. 1955. Serological reactions against egg antigens as an aid in the evaluation of therapy in schistosomiasis. *Am. J. Trop. Med. & Hyg.* **4**, 908-912.

Oliver-Gonzalez, J., Pratt, C. K. 1944. Skin and precipitin reactions to antigens from cercariae and adults of *Schistosoma mansoni*. *Puerto Rico J. Publ. Health & Trop. Med.* **20**, 242-248.

Ozawa, M. 1930. Experimental studies on acquired immunity to schistosomiasis japonica. *Jap. J. Exp. Med.* **8**, 79-84.

Papirmeister, B., and Bang, F. B. 1948. The *in vitro* action of immune sera on cercariae of *Schistosoma mansoni*. *Am. J. Hyg.* **48**, 74-80.

Pellegrino, J. The intradermal test in the diagnosis of schistosomiasis. (Unpublished).

Pellegrino, J., and Macedo, D. G. 1955. A simplified method for the concentration of cercariae. *J. Parasitol.* **41**, 329-330.

Pellegrino, J., and Memoria, J. M. P. and Macedo, D. G. 1957. Quantitative aspects of the intradermal test with cercarial antigen in schistosomiasis. *J. Parasitol.* **43**, 304-307.

Pesigan, T. P., Pangilinan, M. V., Saniel, V. G., Garcia, E. G., Borzon, T. C., and Putong, P. B. 1951. Field studies on the treatment of schistosomiasis japonica with Nilodin. *J. Philip. Med. Assoc.* **27**, 242-247.

Pifano, F. 1953. La Resistencia del Huesped Vertebrado a les re-infecciones por el *Schistosoma mansoni*. *Rev. Sanid. y Assist. Soc.* **18**, 767-781.

Pifano, F., and Ron Pedrique, M. 1957. La cercaria—reaccion de Vogel y Minning en el diagnostico de la schistosomiasis mansoni. *Ztsch. Tropenmed. u. Parasitol.* **8**, 203-207.

Pillemer, L. 1954. Properdin system and immunity I. Demonstration and isolation of new serum protein, properdin, and its role in immune phenomena. *Science* **120**, 279-285.

Pratt, C. K., and Oliver-Gonzalez, J. 1947. Intradermal reactions to fresh and stored antigens prepared from cercariae. *Puerto Rico J. Publ. Health & Trop. Med.* **22**, 254-256.

Price, H. F. 1931. Life history of *Schistosomatium douthitti* (Cort). *Am. J. Hyg.* **13**, 685-727.

Raffel, S. 1953. *Immunity, Hypersensitivity, Serology*. Appleton-Century-Crofts, Inc. 531 pp.

Ritchen, J. 1955. The fevers of Africa. 1. Bilharzial fever. *Cent. Afr. J. Med.* **1**, 37-42.

Ritchen, J., and Gelfand, M. 1954. Katayama disease, early toxemic stage of bilharziasis with a report of a case showing pulmonary infiltration. *Brit. Med. J.* **1**(4876), 1419-1420.

Robinson, D. L. H. 1956*a*. A routine method for the maintenance of *Schistosoma mansoni in vitro*. *J. Helminthol.* **29**, 193-202.

Robinson, D. L. H. 1956*b*. The behavior of young and adult *Schistosoma mansoni* in immune serum. *Trans. Roy. Soc. Trop. Med. & Hyg.* (correspondence) **50**, 105-106.

Schwetz, J. 1955. Sur L'infection naturelle des rats domestiques (*Rattus rattus*) por *Schistosoma mansoni* en Afrique centrale. *Bull. Soc. Path. Exot.* **48**, 182-185.

Schwetz, J. 1956. Sur l'immunité clinique dans les bilharziases humaines. *Bull. Soc. Path. Exot.* **49**, 52-56.

Schneider, M. D., Radke, M. G., and Coleman, M. T. 1956. Immunologically reactive substance from *Schistosoma mansoni*. Exp. *Parasitol.* **4**, 391-397.

Schneider, M. D., Radke, M. G. 1957. Further observations on a complement fixing substance from *Schistosoma mansoni*. *Proc. Med. Soc. Wash.* **24**, 137-139.

Senterfit, L. B. 1953. Immobilization of *Schistosoma mansoni* miracidia by immune serum. *Soc. Exp. Biol. & Med.* **84**, 5-7.

Sherif, A. F. 1956. A new intradermal antigen for the diagnosis of schistosomiasis. *Ann. Trop. Med. & Parasitol.* **50**, 105-112.

Short, R. B. 1952. Sex studies on *Schistosomatium douthitti* (Cort, 1914) Price, 1931 (Trematoda: Schistosomatidae). *Am. Midland Nat.* **47**, 1-54.

Skrjabin, K. I. 1951. Trematodes of animals and man. *Principles of Trematodology*. Vol. 5, Moskow & Leningrad. Izdatelstov. Akademi Nauck SSSR, 624 pp. (In Russian).

Smith, D. A. 1955. Parasitic infections and nutrition. *Vitamins & Hormones*, **13**, 229.

Standen, O. D. 1950. The concentration of cercariae of *Schistosoma mansoni* for the preparation of cercarial antigen. *Trans. Roy. Soc. Trop. Med. & Hyg.* **43**, 527-530.

Standen, O. D. 1952. The *in vitro* effect of normal and immune serum upon the cercariae of *Schistosoma mansoni*. *J. Helminthol.* **26**, 25-42.

Stirewalt, M. A. 1953. The influence of previous infection of mice with *Schistosoma mansoni* on a challenging infection with the homologous parasite. *Am. J. Trop. Med. & Hyg.* **2**, 867-882.

Stirewalt, M. A., and Evans, A. S. 1953. An unsuccessful attempt to protect mice against *Schistosoma mansoni* by transfer of immune rat serum. *Proc. Helm. Soc. Wash.* **20**, 15-19.

Stirewalt, M. A., and Evans, A. S. 1955. Serologic reactions in *Schistosoma mansoni* infections. 1. Cercaricidal, precipitation, agglutination and CHR phenomena. *Exp. Parasitol.* **4**, 123-142.

Stirewalt, M. A., Kuntz, R. E., and Evans, A. S. 1951. The relative susceptibilities of the common-used laboratory mammals to infection by *Schistosoma mansoni*. *Am. J. Trop. Med.* **31**, 57-82.

Sussenguth, H., and Kline, B. S. 1944. A simple rapid flocculation slide test for trichinosis in man and in swine. *Am. J. Clin. Path.* **14**, 471-484.

Taliaferro, W. H. 1929. *The Immunology of Parasite Infections*. John Bale, Sons and Danielsson, Ltd., London.

Taliaferro, W. H. 1940. The mechanism of acquired immunity in infections with parasitic worms. *Physiolog. Rev.* **20**, 469-492.

Taliaferro, W. H. 1943. Antigen-antibody reactions in immunity to metazoan parasites. *Proc. Inst. Med. Chicago* **14**, 358-368.

Taliferro, W. H., Hoffman, W. A., and Cook, D. H. 1928. A precipitin test in intestinal schistosomiasis (*S. mansoni*). *J. Prev. Med.* **2**, 395-414.

Taliaferro, W. H., & Taliaferro, L. G. 1931. Skin reaction in persons infected with *Schistosoma mansoni*. *Puerto Rico J. Publ. Health & Trop. Med.* **7**, 23-35.

Thillet, C. D., and Chandler, A. C. 1957. Immunization against *Trypanosoma lewisi* in rats by injections of metabolic products. *Science* **125**, 346-347.

Thompson, J. H. 1954. Host-parasite relationships of *Schistosoma mansoni*. *Exp. Parasitol.* **3**, 140-160.

Thorson, R. E. 1953. Studies on the mechanism of immunity in the rat to the nematode. *Nippostrongylus muris*. *Am. J. Hyg.* **58**, 1-15.

Thorson, R. E. 1954. Absorption of protective antibodies from serum of rats immune to the nematode *Nippostrongylus muris*. *J. Parasitol.* **40**, 1-4.

Tubangui, M., and Masilungen, A. 1936. Studies on the cercaricidal property of the sera of vertebrate animals. *Philip. J. Sci.* **60**, 393-398.

Vogel, H. 1948. Fiat review of German science 1939-1946. *Trop. Med. & Parasitol.* Editor, E. G. Nauck.

Vogel, H., and Minning, W. 1947. Über die Einwirkung von Brechweinstein, Fuadin und Emetin auf *Bilharzia japonica* und deren Eier in Kaninchenversuch. *Acta Tropica* **4**, 21-56, 97-116.

Vogel, H., and Minning, W. 1949*a*. Hüllenbildung bei Bilharzia-Cercarien im Serum Bilharzia-infizierter Tiere und Menschen. *Centralbl. f. Bakteriol.* **153**, 91-105.

Vogel, H., and Minning, W. 1949*b*. Weitere Beobachtungen über die Cercarienhüllenreaktion, eine Seroprezipitation mit lebenden Bilharziacercarien. *Zeitsch. f. Tropenmed. u. Parasitol.* **1**, 378-386.

Vogel, H., and Minning, W. 1953. Über die erworbene resistenz von *Macacus rhesus* gegenuber *Schistosoma japonicum*. *Ztschr. Tropenmed. u. Parasitol.* **4**, 418-505.

Watts, N. P. 1949. Prophylactic use of schistosomal antigen. *J. Immunol.* **62**, 183-192.

White, P. C., Jr., Pimentel, O.. Garcia, F. C. 1957. Distribution and prevalence of human schistosomiasis in Puerto Rico in 1953. *Am. J. Trop. Med. & Hyg.* **6**, 715-726.

Williams, F. E. 1947. The complement fixation reaction in Asiatic schistosomiasis employing cercarial antigen (*Schistosoma spindale*) *Trans. Roy. Soc. Trop. Med. & Hyg.* **40**, 421-434.

Wright, W. H., Bozicevich, J., Brady, F. J., and Bauman, P. M. 1947. The diagnosis of *Schistosomiasis japonica* V. The intradermal and serological tests. *Am. J. Hyg.* **45**, 150-163.

Yolles, T. K., Moore, D. V., De Giusti, D. L., Ripsom, C. A., and Meleney, H. E. 1947. A technique for the perfusion of laboratory animals for the recovery of schistosomes. *J. Parasitol.* **33**, 419-426.

Yoshimoto, M. 1910. Ueber die Komplement Bindungsreaktion bie der Schistosomum Krankheit in Japan. *Zeitschr. Immunitäts. forsch. u. Exp. Therap.* **5**, 438-445.

THE INDUCTION OF SELF-CURE AND PROTECTION, WITH SPECIAL REFERENCE TO EXPERIMENTAL VACCINATION AGAINST *HÆMONCHUS*

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Foreword

BEING the concluding speaker on this symposium, I shall be allowed to pause a moment, not alone to express again my (and our) appreciation for the opportunity to share in the program, but to add my (and our) congratulations to Rice Institute for two reasons:

First, because of the new facilities for biology teaching and research. We have a way of thinking of physics, chemistry and biology together. But physics and chemistry are in the real heyday of productive activity at this interglacial moment in the so-called ascending spiral of civilization. Biology is only on the threshold, and yet eventually its voice must be heard above the others. Anthropocentric man is first of all a living organism, and from every biological angle needs more understanding of himself and his associated hundreds of thousands of species. So, as has been stated before on this weekend, these new facilities will help in significant and timely fashion to equip those who help to find the biological answers.

The second congratulation to Rice is for Dr. Chandler himself. This transplanted New Jerseyite, with a gift for finding biologically interesting and workable projects (imagine it—from bacteria in cutting compounds, through acquired immunities, to the anatomy of *Mesocestoides,* et cetera) has headed and catalyzed one of the—shall I say three?—most stimulating laboratories of parasitological research and training in this country. After extricating himself early from the momentarily unresolvable quagmire of hookworm in man, he has tenaciously, resourcefully and in good humor probed problems cognate to central puzzles in parasitology. The work of himself and his students has pushed back the limits that fenced in the field of host-parasite relations. And he has with lucidity presented his ideas to an international audience of young and old in the subject, to their profit in understanding some of the intricate interdigitations of the phenomenon of parasitism.

Not only Rice, Houston, and Texas each has a right to be proud of him, but the parasitological fraternity of the whole world—if I may make bold to speak for it—shares that pride and affection.

Introduction

It was my original plan in this paper to undertake an up-to-date review of the status of our knowledge of acquiring resistance, or acquiring immunity, or "self-cure and protection," against the gastro-intestinal nematodes, especially of large mammals. The review was not to have been oriented toward detailed immunologic phenomena such as discussed by my colleagues. It was rather to have been concerned with overall net effects of interplay between worm and host, in terms of long-run survival and well-being of both species. And it was to have had the aim of evaluating any encouraging hints or leads toward developing a more productive approach than the present orthodoxy, against such entities as hookworm, ascaris and whipworm parasitism in *Homo sapiens.*

If it is a reasonable and defensible calculation that these three together encompass two-thirds of the helminthic infections in man (Stoll, 1947), so it may be conservatively concluded they account for more than half the verminous miseries of mankind. For none of these three is there now a program to be mentioned in the same breath with that against malaria, of which you heard Thursday evening from Doctor Russell. Yet it would seem to be among the simplest of prophecies that the future will find means, besides the ones we practice today, of breaking the grip that these worms and man have on each other. Such means, one predicts, will involve bolstering the capacity of the host to self-cure and protect himself, through aiding him antigenically, nutritionally, and anthelmintically—each or all three, perhaps even as simply as by supplying him iodized salt or fluoridated water. What more reasonable then, than to inquire whether there is in present knowledge of worm-host relationships, or, if you will, just beneath the surface of such knowledge, something to contribute to a shortening of that future?

Instead, however, I shall present an experimental study that has no immediate interest in the human problem, but is concerned with vaccination against *Haemonchus contortus.* It was undertaken shortly before the war, interrupted by it, and has hitherto been reported only in abstract (Stoll, 1942). The post-war sequel of the closing of the Princeton laboratory of the Rockefeller Institute has so far made it inappropriate to explore the problem further.

A somewhat better picture of the purely *Haemonchus*-sheep interrelationship is available for orientation now than the one in 1941 when this vaccination study began. Let me refer you particularly to J. S. Andrews (1942) and Stewart (1950 a, b, 1953, 1955). As a preliminary to it may I review briefly the conception one had achieved by about the time it was initiated.

In 1927 a first experiment had been set up, which grouped what were

then four novel factors for furthering the understanding of how a nematode-large host system interacted, in this case *Haemonchus* and sheep. These factors were the availability of lambs reared helminth-free by Smith and Ring (1927), their infection established solely with *Haemonchus contortus,* their exposure to reinfection on a limited pasture area, and the visualization of their infections as a continuing process by means of egg counts—a sort of X-ray time-lag movie of their changing parasitic status. What came about can appropriately be re-stated at this time in the words used in my initial report before the Helminthological Society of Washington on 17 March, 1928. "This type of study resulted in the rather striking discovery of a self-cure phenomenon on the part of the hosts, evidently through elimination of the parasites. Moreover, animals which had gone to a self-cure were protected almost completely against reinfection. The possibility was advanced that perhaps certain special conditions of the experiment produced this effect, although there are numerous anomalous observations in the literature bearing not only *Haemonchus* infection, but on other gastro-intestinal nematodes as well (see for instance Herrick, March 1928, Am. J. Hyg.) which are most easily harmonized with this conception of the intervention of self-cure and protection on the part of the host. The more likely possibility, therefore, is that there occurs, in nematode infections of the type of *H. contortus,* the development of a resistance which is independent of an age resistance per se, and which acts to protect the host to the same end (and which may or may not have the same modus operandi) as obtains for typical bacterial infections." (Stoll, 1929a).

The term, "self-cure and protection" summarized both a logical and biological definition of what had happened. Some new expression had seemed called for, because immunity versus worms was not then in the language of parasitologists. Even my chief, Theobald Smith, expressed skepticism that the result of the experiment was due to acquired immunity. He cautioned that it was more apt to be due to an abnormal worm strain, or to lambs abnormal because bottle-fed.

It is helpful to date this period of change in our thinking, which in a way is well-marked by the fact that Taliaferro (1929) in his "The Immunology of Parasitic Infections," was able to cover the subject of "Acquired Immunity in Infections with Metazoan Parasites" in just 2 pages of a 400-odd page book.

Nowadays we take for granted a host will develop protective response to the presence of a worm species, and we would ask for evidence to prove exceptions. Then, however, there was little or no traffic in the idea. The reversal in regard to it is sufficiently distinct that it is as if professional parasitologists themselves underwent some sort of self-cure and protection against the *absence* of the idea.

Let me illustrate the earlier stage thrice. First illustration. With, among others, Ackert, Augustine, Cadbury, Grant, Hausheer, Hill, Oldt, G. C. and F. K. Payne, Riley, Schapiro, Sweet, and Tseng, I had been associated with Cort during a six-year period of hookworm studies in the Caribbean, China, Panama and Baltimore, in which none of us was led to propose the idea of an acquired immunity being involved in the host-parasite relations we observed. Nor in the great anti-hookworm programs of the quarter-century then ending had other workers interpreted puzzling phenomena they encountered, where *Necator* and *Ancylostoma* met man, as involving anything much more than such forces as *Drosophila* meets in a milk bottle. Thus Sawyer (1925) did not suggest that the problem of acquired immunity had anything to do with the "factors that influence the rate of increase of hookworm infection." Later, from his dog hookworm work, Herrick (1928) found increased resistance after reinfection as one of the factors involved in his results; and Fülleborn, Dios and Zuccarini (1928) and Fülleborn (1929), following hookworm studies in the Argentine, more frankly raised "the question of immunity." However, my friend Chandler seems not to have brought forward any such possibility in either his summary paper on hookworm in India (1928) or on "The rates of loss and acquisition of hookworms" (1929). Three years afterward "The temptation to speculate along these same lines on human hookworm disease is too tempting to resist" was noted by Cort (1932) and in 1940 Cort and Otto observed "although we know of no clear-cut demonstration of the presence of a specific immunity in man acquired through previous infection with hookworms, there is a considerable body of evidence that strongly suggests that host reactions must play an important role in the regulation of human hookworm infection." J. M. Andrews (1942) reemphasized these possibilities, but except for epidemiological implications, the first (and shall I say only?) evidence from man appears to be that furnished by Sheldon and Groover (1942), of Sarles' type precipitates in serum, which seemed so irregular as to be discouraging. There has not been much added since. The situation in relation to "immunity against canine hookworm disease" has been well summarized by Otto (1948).

Second illustration. Faust and Meleney (1924) in their classical monograph on schistosomiasis japonica mentioned in passing, but omitted from the chapter reference list, the article by Fujinami (1916) on "erworbene Immunität." This summary in German of Fujinami's striking experiment and post mortem findings on the development of acquired immunity in the horse against *Schistosoma japonicum,* constitutes the first competent evidence encountered on the subject in relation to typical worm parasites.

Third illustration. Sandground (1928) in "some studies on susceptibility, resistance and acquired immunity to infection with *Strongyloides stercoralis* in dogs and cats" ended his conclusions by affirming that the devel-

opment of the immunity he observed "is an index of the biological *maladjustment* of the parasite to the host." (Italics mine). This point of view was further set forth in a review article (Sandground, 1929).

Perhaps one should add a fourth illustration—the neglected experiments of Ducas (1921) on *Trichinella spiralis.*

In such a climate it seemed prudent to me (Stoll, 1928, 1929b) to follow Stiles wise rule for taxonomy, that if you've found something and cannot be sure of its identity, give it a new name. His thesis was that it is preferable to have an extra synonym than to cover too much territory with one nomen.

To what degree are "acquired immunity" and "self-cure and protection" synonymous? Stewart in Australia has particularly applied himself to resolving the question for the *Haemonchus*-sheep system with the aid of complement-fixation studies, and if you are interested in it, his two papers on "The Nature of the 'Self-Cure' Phenomenon" (1953, 1955) become required reading. In any event self-cure and acquired immunity now exhibit so many features in common that we are not apt to be led astray if we use the expressions somewhat interchangeably.

To return to the Princeton sheep and *Haemonchus.* It was possible to confirm the type of results of the 1927 field study in a new reinfection experiment the following season. Four sheep, aged one and two years, were placed in a fenced one-quarter-acre pasture enclosure. One, lightly infected, seeded the area with *Haemonchus* eggs, and all four became subject to re-infection as they grazed.

You recall that *Haemonchus contortus* has a life cycle substantially like hookworm, with the exception that infection of the host results not from skin penetration by the infective third-stage larvae, but by their ingestion on forage, and that there is no wandering in the adult host, but mature parasitic development on the mucosa of the abomasum, the fourth or true stomach of ruminants. Damage from this parasite is referable to the loss of blood resulting from the feeding habits of the worms (Fourie, 1931; Martin and Ross, 1934; J. S. Andrews, 1942).

In these observations net damage to the host is attested by the well-being or death of the sheep. Increase or decrease of the worm population is inferred from the rise and fall of the egg count, with final autopsy checks.

In the 1928 experiment (Stoll, 1940a), one animal (previously tested in an indoor reinfection experiment) showed little rise in egg count. The other three, with a previous history of just-detectable *Haemonchus* infection, were now subjected to serious reinfection for the first time. One of these exhibited no substantial egg count, another developed an acute infection but became immunized and discharged it, and the last, after a fulminating infection with almost the theoretical additive curve of in-

creased egg output, died with a worm load of over 40,000 *Haemonchus*. No other worm species was involved.

These results did two things. They attested the pathogencity of the worm strain; and, pertinent to today's consideration, they epitomized what, in retrospect, might be labelled characteristic reactions of pure *Haemonchus* infections as observed in sheep.

The later history of the three animals surviving the 1928 season's exposure to reinfection is equally instructive. They were introduced to a regular pasture flock of sheep which contained numerous species common in New Jersey. Besides a predominant *Haemonchus* infestation there were present gastro-intestinal nematodes of the genera *Bunostomum, Capillaria, Chabertia, Cooperia, Nematodirus. Oesophagostomum, Ostertagia, Strongyloides, Trichostrongylus* and *Trichuris,* as well as lungworms and cestodes. Each of the three animals was followed until its death, none, of course, ever receiving anthelmintic treatment.

One ewe, on pasture demonstrated to be heavily infested through the fact that haemonchosis developed in susceptible animals, withstood two seasons. Each spring she showed some rise in egg count, the first season during the period a lamb was suckling, the next in the couple of months before a lamb was born. During the heaviest reinfection periods, summer and fall, she showed no rise the first season, but developed an overwhelming infection at the end of the second season and at death was found to harbor about 7,500 worms, of which approximately 7,000 were *Haemonchus.* On its face this looks like poor protection, and that may be true. However, this ewe had been twice injured by dogs, in one case receiving a broken leg, in the other severe head injuries including the loss of an ear. At autopsy it was found that the head injury had disturbed the set of her jaw so that the teeth did not meet properly. Thus a ridge of high, sharp points developed which eventually prevented rumination. Diagnosis: Emaciation due to food deprivation, with secondary haemonchosis. This would be a perfect case for Whitlock's (1949) thesis.

In a ram there were two subsequent egg count rises, one evidently due to the drain of breeding the flock, a characteristic result with rams. The net result of reinfection in seven successive pasture years, following his first *Haemonchus* exposure, was good, with no sign of essential damage. Then, challenged with a single dose of a quarter million *Haemonchus* larvae (about 4,500/kg), there was no resulting change in egg count; when killed a few months later this ram showed about 500 worms, counting all species. He was to be regarded as adequately immune to *Haemonchus.*

The last animal, a ewe, season after season underwent pasture reinfection conditions with so little infection acquired as to fail sometimes to show a single egg on floatation examination. Year after year she produced vigorous lambs, once a single, twins five times, triplets once. Near the

end of her 8th helminthic reinfection season she developed an acute case of hemorrhagic septicemia (bacteriologically confirmed), and died in a few days after being discovered ill. At autopsy less than 50 worms, involving several species, were found.

It was, of course, possible to repeat the observation on self-cure and protection, or oppositely, a fatal result, with known doses of larvae in indoor experiments.

We had, then, under conditions of exposure to infection on pasture or indoors, sheep hosts undergoing self-cure and protection, against a pathogenic strain of *Haemonchus contortus* capable of producing fatalities; furthermore, these immunized animals could successfully stand exposure to the mixed nematode species on New Jersey pastures year in and year out without treatment (Stoll, 1936a, 1940b).

Studies around the world on ovine and bovine gastro-intestinal nematodes have been made mostly on hosts exposed to just such mixed infections, usually with *Haemonchus* and one or two other species dominating the picture. In trying to untangle the complex problem thus presented by the sheep host reacting to numerous worm species more or less simultaneously, no one has shown more realism than Tetley (1949) in his masterly "Rhythms in nematode parasitism of sheep." Fruitful observations on all or part of the complex, primarily from the epidemiologic standpoint, have been made by many workers, including Ross (1932), Kauzal (1933), Gordon (1948, 1949, 1950), and Stewart (1950 d) in Australia; Taylor (1934 a, b), and Crofton (1955) in England; Morgan, Parnell and Rayski (1951) in Scotland; Swales (1940) in Canada; and Marsh (1936), Hawkins et al. (1944), Whitlock (1949, 1955), and Levine et al. (1956) in this country. Also by Roberts, O'Sullivan and Riek (1952) on cattle in Australia.

Eventually, of course, the mosaic of protective mechanisms in the domestic ruminants will need to be patiently unravelled through study of individual worm-host systems. There are already a number of positive experiments indicating specific immune responses to other species than *H. contortus*. In addition such responses may be allowably inferred from certain investigations designed primarily to improve animal husbandry, to demonstrate life histories, or to test pathogenicity of given species with huge challenge infections. The impact of some of the latter on the unprepared host suggest a resemblance to the impact of an automobile driven at 60 miles an hour when it is ordinarily in operation at a reasonable fraction of such speed, unless and until both driver and car are conditioned to potentially cataclysmic events. We would be in error to believe there are any host resistances that cannot be broken, including potential immunities before they get into gear. Learning to elicit im-

munities may require more subtlety and difficulty than to break them. If, however, they exist, biological interest in the host will continue to encourage their discovery.

It should be added ruminants are not always available, or easy, to study under adequately controlled experimental conditions. Nevertheless, as indicated, a view is emerging of specific acquired immunities in ruminants resulting from the parasitism of members of many of the genera earlier mentioned. They are illustrated, for instance, in the observations of Mayhew (1940), Sprent (1946), and Lucker and Neumayer (1946) for *Bunostomum;* Ross and Kauzal (1933), and Threlkeld (1948) for *Chabertia;* Andrews (1939 b) for *Cooperia;* Tetley (1935), Kauzal (1937), Kates and Turner (1953), and Herlich (1954) for *Nematodirus;* Mayhew (1940), Andrews and Maldonado (1943), and Goldberg (1951) for *Oesophagostomum;* Threlkeld and Downing (1936), Stewart, Miller and Douglas (1937), and Porter and Cauthen (1946) for *Ostertagia;* Vegors (1954), and Turner (1956) for *Strongyloides;* Mönnig (1927), Andrews (1939 a), Stewart, (1950 c), Gibson (1952), and Stewart and Gordon (1953) for *Trichostrongylus;* and, perhaps also to be included, Seddon (1931) and others for *Moniezia expansa. Capillaria* and *Trichuris* spp. appear to be undefined in this relation.

In my own work all attempts continued to fail to induce a state of resistance to *Haemonchus* or to diagnose its presence, by means other than reinfection itself. This experience with sheep thus did not parallel that of Chandler (1932) in measurably protecting rats against *Nippostrongylus muris;* or of Sheldon (1937) in similarly protecting rats against *Strongyloides ratti* by subcutaneous injections of infective larvae heat-killed at 55-58°C.; or of Watt (1943) inducing some immunity in rats against *N. muris* with Seitz-filtered extracts from dried larval or adult worms; or of McCoy (1935) in partially protecting rats against *Trichinella spiralis* by intraperitoneal injections of heat-killed larvae; or of Spindler (1937) inducing some resistance to intestinal trichinosis in small laboratory animals "by feeding metabolic products of encysted trichinae."

Nor could I convincingly demonstrate Sarles' (1938) precipitates forming at the oral or other apertures of exsheathed infective *Haemonchus* larvae in serum from immune or hyperimmune sheep, although such precipitates did form in the serum of guinea pigs 25 days after receiving *Haemonchus* larvae intraperitoneally. Such precipitates also formed in the serum of calves, especially if they were *Haemonchus*-positive. Hawkins and Cole (1945) have since reported such precipitates forming in the serum of sheep with mixed nematode infections. Stewart's (1950 a) complement fixation test was still a long way off.

Nor could I observe any immobilization or debilitating effects on in-

fective *Haemonchus* larvae immersed in serum from immune sheep, even though no precipitates were formed. Nor, of course, did parenterally administered extracts made from larval or adult *Haemonchus* result in any protective immunizing effect against infection in sheep. Later Stewart (1950 b) also found these fruitless. Mayhew (1944, 1949 b) has reported similar negative results in tests with *Haemonchus* in the bovine host, although he had earlier been able to develop immunity in calves by oral infection with live larvae (1941), and later to confirm it (Mayhew, 1953).

May I digress a moment to note that in 1928 it had been possible also to show that self-cure and protection was not a function alone of the blood-sucking *Haemonchus,* but could be demonstrated (Stoll, 1932), under conditions of natural reinfection in *Oryctolagus cuniculus,* the domestic rabbit, against *Trichostrongylus calcaratus.* A later investigation by Sarles (1932) indicated that this worm-host system was amenable to controlled experimental results that had many of the aspects of the overall picture in sheep. This was particularly gratifying at the time because Sarles came to Princeton in skeptical mood about the reaction, his work on dog hookworm in Baltimore having led him rather to the conclusion that age immunity was the significant factor. Indeed, this skepticism held up to the next to the last measured worm dose he gave his first rabbit prior to its reacting against the worms, the rabbit having shown unmodified susceptibility up to then. It is such a result and the somewhat parallel experience of Otto, Kerr and Landsberg (1937) with the dog hookworm, that makes one wonder whether Beaver's observation on the apparent failure to immunize a human volunteer against hookworm may not have stopped one or two immunizing doses too soon (Beaver, 1945).

For more information on the experimental work of this period and later, which engaged the attention of a number of investigators, and with special reference to the nematode parasitisms of small mammals, I refer you to such reviews as those in Chandler (1937, 1948), Clapham (1933), Culbertson (1938), Taliaferro (1940, 1943), and Wetzel (1952).

The bulk of this work was indicating that while acquired immunities could be secured against nematodes, it had been successful primarily as a result of the process of infection or reinfection. Against nematodes vaccination procedures had not developed much promise. At this point, then, it seemed probable that the protective immunizing effect was being produced in the host against the secretions and excretions of living worms, as the current studies of Thorson (1953, 1954) with *Nippostrongylus muris,* and Campbell (1955), Chute (1956), and Chipman (1957) with *Trichinella spiralis* have now demonstrated for those two species.

But if secretions and excretions of the living worms were to be em-

ployed, how make them available in useful quantities as a vaccine? The possibility that a vaccine (in my definition, an inoculable immunizing agent) might be had in the accumulated metabolic products of the worms was one of the factors that lent encouragement to attempting the in vitro culture of the parasitic stages of *Haemonchus*. This Glaser and Stoll (1938) were able to carry only part way. The axenic work did, however, among other things, give practice in the sterilizing or axenizing of normally-grown infective *Haemonchus* larvae (Glaser and Stoll, 1940). Indeed, producing such larvae that had been obtained from fecal cultures and subsequently rendered bacteria-free to the degree that several hundreds or thousands in a broth tube sterility test would fail to show any contaminative growth, was turned into a routine laboratory procedure. The availability of this new biological reagent, namely bacteria-free infective larvae, permitted the final demonstration that *Haemonchus contortus* does not wander in the body of the sheep host (Stoll, 1943). And this led naturally to the next idea, that such bacteria-free, non-wandering *Haemonchus*, might have the capacity to elicit a host reaction favorable to protection against similar parasites later reaching the host by the normal oral route.

The remainder of my remarks are concerned essentially with a partial test of that idea. They can appropriately be introduced in the recent words of Chandler (1953), "If functional immunity is due, even if only in part, to reaction against metabolic products of parasites, it would seem that investigation of the possibilities of immunization by the use of metabolic products might be a fruitful field of investigation." In these experiments bacteria-free third-stage larvae of *Haemonchus contortus*, that do not wander in the usual nematode meaning,are emplaced and allowed to deliver metabolites as living organisms parenterally, for an ultimate effect brought to bear at an enteral target site.

Material and Methods

The sheep hosts here reported were taken from their dams and bottle-raised (Smith and Ring, 1927). Reared indoors they were demonstrated to be helminth-free by repeated floatation examinations, and by Baermann isolations of fecal cultures, up to the time of entering the experiments. The two bull calves had nursed from their dams for two days, and then were weaned and fed milk from a pail. No helminth eggs were detected in either of them until one became positive for *Haemonchus* in the course of the experiment.

Live larvae for parenteral injection and oral test were cultured from fecal pellets of seed animals (sheep) with pure *H. contortus* infections. Oral test doses of larvae were sheathed infective 3rd stages, washed, and

administered by pipette in a few ml. of tap water to the back of the mouth held open by an attendant.

Larvae for injection were similarly grown, then exsheathed and sterilized by the method of Glaser and Stoll (1940). They were demonstrably bacteria-free before use. Injections were in a few ml. of tap water. Subcutaneous injections were usually given under the skin in the wool-free areas on the inner aspects of the hind legs near the groin, half the dose on each side.

On a given day and in a given experiment, recipients of challenge doses received fair samples from a single pool of sheathed larvae, and those injected received fair samples from a single pool of sterile exsheathed larvae.

For occult blood tests of the feces the method of Thevenon and Rolland was employed, as described by Arnaud (1932) in his study of hookworm-infected natives near Leopoldville, Belgian Congo. This makes use of an alcoholic solution of pyramidon (amidopyrine) with a few drops of glacial acetic acid and hydrogen peroxide. The violet color developed varies in intensity with the amount of blood present. The "unknown" tested was a standard amount of the aqueous fecal suspension prepared for the floatation examination for *Haemonchus* eggs.

Jugular blood drawn into oxalated tubes, or, in anemic animals, blood from the ear obtained by needle prick, was used for hemoglobin determinations (Talquist) and stained smears (Wright's).

Dilution and floatation egg counting methods have been earlier described (Stoll, 1930).

Vaccination Experiments with Sheep

In three experiments designed to determine whether *Haemonchus* larvae administered by the IP (intraperitoneal) or SC (subcutaneous) routes would assist development of self-cure and protection against single challenge oral infections, 13 sheep were utilized. These had been born in early spring and, when given their oral test doses of normal, sheathed, infective *Haemonchus* larvae in Sept. to Feb., 4 were 7 months, 4 were 8-9 months, and 5 were 11-12 months old. The 3 rams, 2 wethers (castrates) and 8 ewes weighed 57 to 101 pounds (25.8 to 45.8 kg) each, and included 3 pair of twins (ram 881 and ewe 882; ram 890 and ewe 891; ewes 899 and 900).

Five animals received IP injections of live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae 7 to 11 weeks before oral test. All showed a successful resistant response to challenge infections, a response that contrasted strikingly with the fatal haemonchosis developing in 2 uninjected controls otherwise similarly handled. No greater resistance appeared in 2 of the animals given 4 injections spaced one week apart, than in the

3 that were given single IP injection of larvae. (In one of the former, ewe 897, the timing of the initial appearance of eggs in the feces gave evidence of entry into the rumen of at least part of the first and fourth injections). Each animal received as challenge a single oral dose of 10,000 to 15,000 larvae per kg. body weight. The IP injections totalled 3600 to 22,000 larvae per kg. body weight.

Five animals received single SC injections of larvae. Two injected 5 weeks before, one injected 9 weeks before, and one injected less than an hour before challenge infection, showed a successful resistant response, again in striking contrast to the severe but, in this instance, non-fatal haemonchosis that developed in the uninjected control No. 892, otherwise similarly handled. The fifth animal, a year-old pregnant ewe No. 891, received the SC injection 3 weeks before oral test, lambed prematurely 3 weeks after the test, and succumbed 3 weeks afterward. The SC injections were 3100 to 4100 larvae per kg. body weight, the single oral challenge doses 15,000 larvae per kg., and the animals a year old at test (except one at 7 months).

The other three animals were given no injections, but were challenged orally in a similar manner.

Following the challenge doses, of the 9 animals successfully protected after IP or SC larvae (that is, all but one of the 10 injected), 6 showed little or no blood loss, as measured either by hemoglobin readings of the circulating blood, or by OBT (occult blood tests) of the feces. In only two did the egg count exceed about one MEPD (million eggs per day), these two reaching, respectively, 2 MEPD, and 5 MEPD, both transiently.

These 6 animals that were best protected by the parenterally emplaced larvae comprised 2 rams and 4 ewes. Ewe 899 had not at any time been positive by OBT, her egg count had barely reached one MEPD, and when autopsied 12 weeks after challenge harbored only 279 *Haemonchus* adults, i.e., about 0.05 per cent of her test dose. This was the only one of the 4 coming to autopsy after parenteral injection of larvae which exhibited sign of the injection sites. Ewe 899 had received SC larvae once, 5 weeks before oral test. Now, 4 months after SC, there was at the site of injection in each groin a single subcutaneous "cyst" measuring about $1\frac{1}{2} \times 1 \times 1$ cm.; each had a thick capsule and caseous contents, indicating probable bacterial contamination at the time of injection.

Ram 890 (twin to ewe 891, the only injected animal that died) after becoming negative to a lightly positive OBT, and to an egg count that reached 5 MEPD on one examination, was killed 14 weeks after challenge. Only 3 *Haemonchus* could be found. This ram had received SC larvae once, one hour before oral test. At autopsy there was no sign of even the exact site of injection.

The second ram, 895, having scarcely exhibited sign of infection by

OBT, and with a transient egg count maximum of 2 MEPD for only one week, was released 2 months after challenge for sale in the war effort.

The other 3 ewes, Nos. 882 (twin of ram 881 mentioned below), 897 and 900 (twin of ewe 899 mentioned above), were returned to the pasture flock 2 to 4 months after challenge, this being January for 882 and 897, and June for 900. These 3 had not at any time since the oral test dose shown a distinctly positive OBT, and their egg counts had scarcely reached one MEPD.

The other 3 injected animals, IP wether 899, IP ram 881, and SC ewe 888, had shown considerable blood loss temporarily after the challenge doses, as indicated by lowered hemoglobins and OBT. However, the *Haemonchus* egg counts, considered by themselves, were neither very high nor prolonged. Their records were as follows:

Wether 889 had 4 IP injections at weekly intervals, and was challenged 4 weeks after the last. He became negative within 10 weeks after oral test, and remained so for 3 weeks both by OBT and egg count, one later examination showing about 4 MEPD. Then he exhibited a "relapse" by again showing a positive OBT and reaching about the same egg count as previously. This manifestation of renewed pathogenicity of residual (and temporarily retarded?) worms, or of a lapse of resistance, lasted all told about 5 weeks. It then ended by OBT and egg counts again becoming negative without intervention by the investigator. Wether 889 was killed a week later to determine whether *Haemonchus* still persisted in his abomasum. Not one worm was found nor was there any trace of the IP injection.

The possibility of contaminative reinfection here as the cause of the "relapse" is remote. Rigid sanitary precautions had been early established by Theobald Smith for routine cleaning of animal quarters at the Princeton laboratory of the Rockefeller Institute. That they were effective in these experiments with *Haemonchus* is attested, for instance, by a helminth-free wether of similar age having been maintained in the unit during this experiment for 15 weeks. He retained a helminth-free status as determined by failure to demonstrate nematode infection even by Baermann isolation of fecal cultures.

IP ram 881 developed the highest egg count, about 6 MEPD on one day, and more blood loss than any of the other injected animals (except ewe 891 that died). Nevertheless, by 8 weeks after challenge he had become negative by OBT, and so remained throughout the succeeding 14 months during which he was examined. Sixteen weeks after challenge, when a barely detectable egg count remained as the result of the challenge dose, this ram was tested with a succession of large oral doses of

infective *Haemonchus* larvae for a period of 16 weeks. On 26 occasions, at intervals of 2 to 7 days, he was given larvae at the rate of 14,000 to 30,000 per kg. body weight, until he had received 15 million larvae. Five days after this series 350 ml. of blood from the jugular (about 13 per cent of blood volume) was withdrawn; the next day he received 500,000 more larvae by mouth, and a week after that 10,500,000 additional in a single dose. Throughout the whole period of his getting these 26 million larvae, and for the 8 months thereafter that he was under examination, he remained untroubled by this rigorous *Haemonchus* assault. He showed a continuously negative OBT, was negative or nearly negative to floatation fecal examination, regained normal hemoglobin values after the setback due to his original challenge oral infection, and gained weight. He was thus demonstrated to be magnificently protected.

SC ewe 888's history of blood loss and egg count following challenge roughly paralleled that of ram 881, but was less pronounced. She was returned to pasture in January, 16 weeks after oral test, as having undergone self-cure.

There had been three fatalities as an immediate result of the experiments. Ewe 891, the 10th injected animal, has already been noted as a pregnant ewe lambing prematurely after an oral challenge infection 3 weeks after SC larval injection. She died 6 weeks after the oral test with a profound anemia when passing about 13 MEPD, and 15,270 *Haemonchus* were recovered from the abomasum at autopsy, i.e., 2.7 per cent of the oral test dose. There was no sign discovered of the SC injections she had received.

The other two fatalities were among the uninjected controls, following oral tests. Ewe 886 succumbed 25 days after receiving 15,000 larvae per kg. orally, and before becoming egg-count positive. Her worm count was 29,130 *Haemonchus,* i.e. 6.6 per cent of the oral test dose. Ewe 901 died 21 weeks after receiving 10,000 larvae per kg. orally, and after a continuously high egg count which exceeded 20 MEPD during most of the period. Her worm count was 9690 *Haemonchus,* i.e. 3.0 per cent of the oral test dose.

The third uninjected control, wether 892, did not succumb after receiving the test infection of 15,000 larvae per kg. orally, but showed typical severe haemonchosis. The egg count reached 14 MEPD the 7th week, and continued high, falling off less than half in the next 10 weeks. As with fatal cases there was a markedly lowered hemoglobin, and strongly positive OBT. By 20 weeks after the oral test the OBT had disappeared and did not reappear, although the egg count remained about 4 MEPD. Then placed in pasture (late June) the egg count fell sharply in about 6 weeks to 0.2 MEPD. When killed 3 months later this wether then had

only 22 *Haemonchus,* but had 15,200 *Trichostrongylus axei* and 120 *Oesophagostomum.* This was 41 weeks after the oral test dose indoors, of which the last 18 weeks had been under out-door conditions exposed to mixed species in pasture.

To summarize these three experiments, of the 10 sheep 7 to 12 months of age that were parenterally injected with live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae, 9 showed evidence of enhanced protection, 6 of them to a high degree, against the challenge of a single huge oral dose of larvae. (The 10th, yearling ewe 891, demonstrated no increased resistance, probably because of the complication of parturition 3 weeks after oral challenge.) Of these 9, the protection afforded to 6 was, as stated, striking, whether judged by blood hemoglobin values, OBT of the feces, egg counts, or the worm counts of the 2 killed for examination. In 3 other animals the protection afforded by the parenteral larval injections was less in degree, but nonetheless effective, one of the 3 autopsied 19 weeks after test being *Haemonchus*-free. In contrast, 3 uninjected controls of similar ages, and handled similarly throughout except for the omission of parenteral injections, developed severe cases of haemonchosis from which 2 died with post-mortem worm counts of 9690 and 29,130 *Haemonchus,* respectively, and the third survived only after a lengthy convalescence.

Of the 9 injected animals, besides the 3 killed for examination, one (ram 881) was not fazed later by a protracted series of oral doses that totaled 26 million larvae in 16 weeks, and 5 were returned to the pasture flock as self-cured. One of these latter, ram 895, was shortly sold and his later history is unknown. The 4 ewes were kept under observation during their initial pasture season, but were given only occasional fecal examinations. These indicated that their reaction to the mixed species on pasture, including *Haemonchus,* was not troublesome, and typical of animals that had undergone self-cure. Two of the ewes lambed the following season, i.e. after their first opportunity to be bred.

Vaccination Experiment with Calves

One experiment was performed to determine whether *Haemonchus* larvae of sheep origin administered parenterally to a calf would assist the development of self-cure and protection against a single, oral challenge infection.

Two Guernsey bull calves, S2120 born 25 April, and S2119 born 21 April (hereafter referred to as A and B), were brought to the laboratory 29 April, placed together in a large stall, and started on 2 pounds of dry milk and one pound of grain mixture per day, together with mixed hay. On 21 May they weighed, respectively, 87 and 113 pounds (39.4 and 51.2 kg.).

The milk was discontinued for both animals on 13 June, 23 days after SC injection of calf A. As this was 19 days before the challenge infections of both calves it is unlikely that the milk feedings played any role in later decreasing their parasitization by *Haemonchus*, such as was observed by Porter (1941).

On 21 May, the smaller calf A, then 26 days old, received subcutaneously 145,000 live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae of sheep origin, representing 3700 per kg. body weight. The larvae, in sterile tap water, were divided into 3 portions of 2 ml. each, for injection sites on the inner aspects of each hind leg, in the region of the groin, and at the right side of the neck. The injection sites showed nothing of interest and were difficult to identify when examined 12 and 22 days later. Calf B was left uninjected.

On 2 July, 6 weeks after the SC injection of A, each calf was given a dose of normal, sheathed, infective *Haemonchus* larvae of sheep origin, administered by pipette into the back of the mouth in about 8 ml. of water. The calves were now 10 weeks old and weighed, respectively, 127 and 165 lbs. (57.5 and 74.7 kg.), each having gained 46% over his weight on 21 May. Calf A received 875,000 larvae and calf B, the uninjected control 1,125,000, each infection representing 15,000 larvae per kg. body weight.

In large single oral doses of larvae given to sheep there is demonstrable some immediate loss of worms that fail to stay in the abomasum. This was also demonstrable for these calves. Fecal samples from both calves, secured 23 hours after oral administration of larvae, showed about 20 larvae per gm. of feces by salt floatation. All these recovered larvae were now exsheathed, and half of them showed activity. As an extra prevention against fortuitous re-ingestion of larvae from fecally-soiled bedding, the animals were transferred to a clean stall 24 hours after the oral dose.

The results of the experiment may be followed by reference to Fig. 1.

Hemoglobin. Talquist readings (dash line, upon circles) on both animals remained at 90-95% for a fortnight after the oral dose. In calf A they were reduced to 70-75% at the 4th week, and then rose again to 90-95% at the 8th week. In control B the readings went lower, to 60-65% in both the 4th and 5th weeks, and then rose to 80-85%.

Occult blood. For calf A all OBT were negative (open circles on base line) except for some "possibly positive" on days 13 to 29 after oral challenge. In control B the OBT were positive in increasing intensity on days 11 to 18 (solid line), stayed high with some variation until day 32, then fell off to become negative on day 37, and remained so. It will be noted in Fig. 1 that the period of 30% fall in hemoglobin in control B coincided with the period of greatest blood loss determined by OBT. Also that the

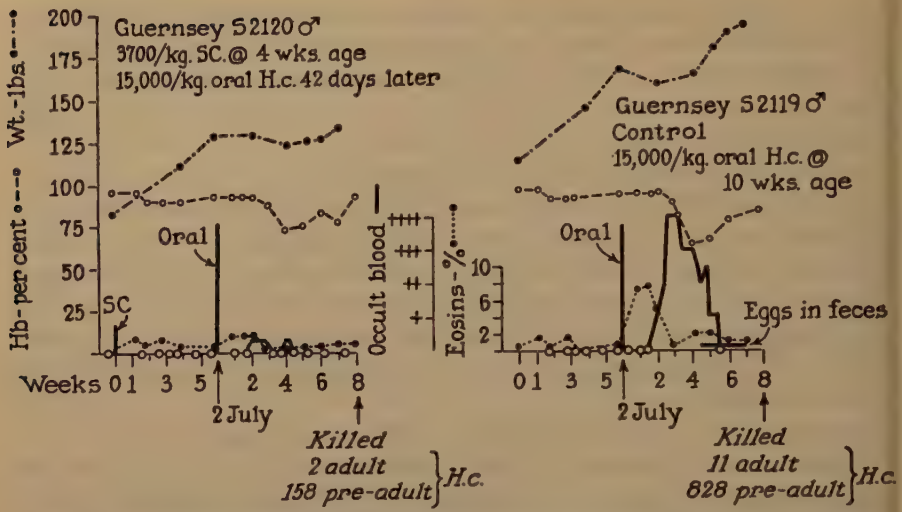

FIG. 1. Result of experimental vaccination of Guernsey bull calf S2120 by single subcutaneous injection of live, exsheathed, bacteria-free 3rd stage *Haemonchus contortus* larvae of sheep origin, and then challenged after 6 weeks with a single oral dose of sheathed infective *Haemonchus* larvae, as contrasted with uninjected control calf S2119 similarly tested. The comparative size of the SC injection (3700 larvae per kg. body weight) and of the oral challenge infections (15,000 larvae per kg. body weight) is indicated by the height of the respective vertical bars. The animals were of similar age, weighed 127 and 165 pounds (57.5 and 74.7 kg). respectively when challenged, and are referred to in the text as calf A and control B.

OBT became, and stayed, negative while eggs in the feces continued to appear for another 10 days.

Eosinophiles. In calf A throughout the experiment the eosinophiles (dotted line) did not rise above 2%. In control B this was true preceding the oral dose, but on days 6 and 11 after it they were at 7 and 7½%. Counts were on 200 leucocytes.

Fecal examinations for Haemonchus *eggs.* Both animals remained negative by floatation examination on weekly samples preceding the oral doses. The positive larval findings the day after the larval oral dose have been noted. Between days 7 and 54 of the post-oral-test period each animal received floatation examinations at 1 or 2-day intervals. For calf A these were uniformly negative in result. For control B they were positive on days 32 to 51 (negative 2 days before and after), shown with heavy line parallel to the abscissa in Fig. 1. Using the empirical formula of 20 times the bovine weight in pounds multiplied by the eggs per gram to represent the eggs per day (Stoll, 1936 b), the egg output per day of control B rose to 25,000 on the 36th day after oral infection, 50,000 on the 37th day, 100,000 on the 39th day, and was at 400,000 on the 41st to 46th

days. It then dropped 99% on the 48th, and was negative the 53rd day. While the absolute size of the counts was small, their numbers described the characteristic configuration of a self-cure curve.

Weights. The calves were weighed just before and at 2, 4, 5, 6 and 7 weeks after the oral test dose of larvae. As the dot-dash line in Fig. 1 shows, both temporarily failed to gain after the oral test, with apparent maximum losses of 3 lbs. for A and 7 for control B. By the end of the 7th week A was at 134 lbs., control B at 192 lbs., respectively, 105% and 116% of the pre-oral-test levels. The temporary damping of the weight curves is doubtless more significant in rapidly growing juvenile animals than the actual amount of change, when account is taken of the initial difference in weight of the two calves.

Autopsy findings. Calf A was killed 57 days after the oral test dose and was normal in appearance. Careful dissection of the subcutaneous areas of larval injection in the groin and neck sites failed to reveal even the exact places the inoculations were made. Thorough worm count of the abomasum showed 2 male adult *Haemonchus* and 158 immature worms about 2-3 mm long.

Control calf B was killed 54 days after the oral test dose and was likewise normal in appearance. Worm count of the abomasum revealed 2 male and 8 female adult *Haemonchus* and 828 immature 4th stages. One adult male was added by examination of the screened contents of the small intestine. Measured in physiological saline after refrigeration overnight the males were 10.3-13.0 mm., the females, all egg-bearing, 13.9-16.6 mm, and a fair sample of the 4th larval stages were 1.3-2.4 mm. long.

Despite the effort made to gain complete worm counts on the calves post-mortem, the fact that so many were pre-adult stages naturally jeopardized the accuracy of examination in a large ruminant, although I think in these instances it was more theoretical than real. Thus while less than one-fifth the number of worms were recovered from previously injected calf A killed 3 days later than control B, the conservative position is that this may not have been a significant difference, even though it is in the right direction.

The worms recovered, as well as the passing of *Haemonchus* eggs by calf B, gave direct proof that *Haemonchus* of sheep origin are infective for calves, although possessing a lower grade of infectivity for the bovine than for the ovine host. This is in accord with the findings of Ross (1931), who also showed that *Haemonchus* of bovine origin infect sheep, as did Stoll (1936 b.). If, however, the determination by Roberts, Turner and McKevett (1954) that ovine and bovine "strains" of *Haemonchus* represent different species is true locally, the possibility needs consideration that some fraction of the *Haemonchus* larvae of sheep origin used in the ex-

periment actually represented the bovine species capable of maintaining residence in sheep.

To summarize, of two Guernsey bull calves of equal age, calf A parenterally injected with live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae of sheep origin, evidenced protection against the challenge of a single huge oral dose of sheathed infective *Haemonchus* larvae of sheep origin administered 6 weeks later, as contrasted with uninjected control calf B similarly challenged. Calf A exhibited minor depression of hemoglobin and negligible occult blood in the feces in contrast to calf B, which showed greater decrease of hemoglobin, with occult blood in the feces for 25 days and in large amount. No *Haemonchus* eggs were detected in the feces of calf A following challenge, whereas control B was egg-count positive for 3 weeks. There was temporary damping of the weight curves in both animals. At autopsy 8 weeks after challenge there were found 160 *Haemonchus* in calf A, 839 in calf B. These differences, showing protection in the injected animal, were more striking in that Calf A at challenge weighed 127 pounds, control B 165 pounds.

Regarding Parenterally Emplaced Haemonchus Larvae

The fact that parenterally-injected, live, exsheathed bacteria-free 3rd stage *Haemonchus* larvae do not wander in the usual nematode fashion has been earlier reported (Stoll, 1943). By indirect evidence, their inability to reach the abomasum was attested in the present experiments by consistently negative floatation examinations of feces for the presence of larvae or ova in the period from the time of injection to two weeks after the oral challenge in 9 animals. The 5 SC-infected sheep received 40 such examinations (3 to 11 each), all negative. In IP injections the hazard of inadvertently injecting directly into the rumen has been mentioned in the instance of ewe 897. In the 4 other animals, one of which received 4 IP injections, the others one IP each, all of 93 floatation examinations (14 to 28 per animal), were negative. By direct evidence, the larvae appear to be trapped at the injection site where they are able to survive only temporarily.

A measure of this period of survival of parenterally-emplaced larvae was furnished by 3 hitherto helminth-free sheep examined at 3, 6 and 12 days after IP injection of larvae. In wether H38, 63 weeks of age and killed 3 days after IP injection, the worms, entangled in small hyaline nodules observed chiefly on the omentum, were for the most part found alive. In wether H85, 21 weeks of age and killed 6 days after IP, a few larvae were observed to be still alive in such nodules, but most were inactive and probably dead. In ram H52, 70 weeks of age and killed 12 days after IP, the nodules had lost their hyaline character and were firm,

were difficult to examine in squeeze preparations under the microscope, appeared well vascularized, and the larvae within them were vacuolated. Peritoneal fluid and washings of the peritoneal cavity in these animals revealed no larvae.

Photomicrographs of a squeeze preparation of a nodule from H38 shows (contrary to an item in Stoll, 1943) that many of the larvae were ensheathed for the first parasitic ecdysis terminating the 3rd larval stage, and at least one larva, already exsheathed, evidenced some development into the 4th larval stage.

Confirmatory observations were made in ram J17, 63 weeks of age. This animal was given SC injections on the inner aspect of the right leg near the groin 7 days before, and similarly of the left leg 3 days before being killed. There was an infiltrated mass of reaction tissue in both sites, firmer and more consolidated in the 7-day area. Photomicrographs of squeeze preparations of both the 3-day and 7-day sites show numerous larvae ensheathed preparatory to the first parasitic ecdysis; on the left side, injected 3 days earlier, some of these larvae have the appearance of having been alive when photographed; on the right side, at 7 days, all are vacuolated and of flaccid appearance.

These observations are direct evidence that the bacteria-free, parenterally-injected worms remain alive for a few days, during which many achieve their first parasitic ecdysis, before being overwhelmed and killed by the local reaction. Ample opportunity is thus afforded for metabolites in the form of secretions and excretions of the worms while alive to be absorbed by the host.

Conclusion

Maurice C. Hall once wrote, "parasitism is not a static and fixed thing." No more, it should be added, is our conception of it. Self-cure and protection against worms did not begin three decades ago. What then began was the recognition, as widespread, of a force that Nature had been generating for untold time to help balance hosts in relation to their worms.

Mobilization by a host of the self-cure and protection mechanism, or of acquired immunity, through the stimulation furnished by specific chemical products of metabolism of a worm parasite, is capable in its ultimate manifestation of preventing residence within the host of even a single helminth producing those specific metabolites. Usually, as happens characteristically with other types of etiological agents as well, something less than complete resistance or extirpation of every parasite is the result. In either event, the capacity of responding to the presence of the living parasite is quickened, or enhanced in degree, as compared to a host that has lacked such a specific biological experience.

It is submitted that experimental vaccination against *Haemonchus* by

live, parenterally-emplaced, non-wandering larvae appears capable of assisting to provide that experience for both the ovine and bovine host.

In the general scheme of things vaccination against worms could conceivably make a place for itself, as have immunities acquired through the normal process of infection and reinfection, and as have nutrition, and anthelmintics, and genetics, although none of these, in present view, can alone take over control of worm-host systems for the total protection of the host.

Moreover, *Haemonchus* is not only gastro-intestinal nematode unable to migrate from a parenteral injection site. Bacteria-free, infective larval stages of *Haemonchus,* and other non-wandering species, may therefore be taken to represent new biological reagents with which to explore further some of the phenomena of immunization against worms.

ACKNOWLEDGEMENTS

The studies here reported were only made possible through the dependable and skillful technical assistance of Patrick Browne, my laboratory associate for 16 years. Advice and cooperation on the animal husbandry phases of the sheep work in the same period were received from E. Raymond Ring, Assistant Superintendent of the Department of Animal Pathology of the Rockefeller Institute. J. H. Hambleton was an additional assistant in the laboratory phases, and James A. Smith and associates had the daily care of the animals. This records for each of them a deep measure of appreciation.

REFERENCES

Andrews, J. M. 1942. Modern views on the treatment and prevention of hookworm disease. *Ann. Inter. Med.* **17,** 891-901.

Andrews, J. S. 1939*a*. Experimental trichostrongylosis in sheep and goats. *J. Agric. Res.* **58,** 761-770.

Andrews, J. S. 1939 *b*. Life history of the nematode *Cooperia curticei,* and development of resistance in sheep. *J. Agric. Res.* **58,** 771-785.

Andrews, J. S. 1942. Stomach worm (*Haemonchus contortus*) infection in lambs and its relation to gastric hemorrhage and general pathology. *J. Agric. Res.* **65,** 1-17.

Andrews, J. S., and Maldonado, J. F. 1943. Some clinical aspects of experimental esophagostomiasis in cattle. *Am. J. Vet. Res.* **4,** 211-225.

Arnaud, R. 1932. Rapport de mission sur l'ankylostomiase dans le secteur du laboratoire de Leopoldville. *Ann. Soc. Belge. Med. Trop.* **12,** 1-34.

Beaver, P. C. 1945. Immunity to *Necator americanus* infection. *J. Parasitol.* **31,** Suppl., 18.

Campbell, C. H. 1955. The antigenic role of the excretions and secretions of *Trichinella spiralis* in the production of immunity in mice. *J. Parasitol.* **41,** 483-491.

Chandler, A. C. 1928. The prevalence and epidemiology of hookworm and other helminthic infections in India. XII. General summary and conclusions. *Indian J. Med. Res.* **15,** 695-743.

Chandler, A. C. 1929. The rates of loss and acquisition of hookworms. *J. Am. Med. Assn.* **92,** 1337-1341.

Chandler, A. C. 1932. Experiments on resistance of rats to superinfection with the nematode, *Nippostrongylus muris. Am. J. Hyg.* **16,** 750-782.

Chandler, A. C. 1937. Studies on the nature of immunity to intestinal helminths. VI. General résumé and discussion. *Am. J. Hyg.* **26,** 309-321.

Chandler, A. C. 1948. Factors modifying host resistance to helminthic infections. *Proc. 4th Inter. Congresses Trop. Med. & Malaria,* Washington. **2,** 975-981.

Chandler, A. C. 1953. Immunity in parasitic diseases. *J. Egyptian Med. Assoc.*, **36**, 811-834.

Chipman, P. B. 1957. The antigenic role of the excretions and secretions of adult *Trichinella spiralis* in the production of immunity in mice. *J. Parasitol.* **43**, 593-598.

Chute, R. M. 1956. The dual antibody response to experimental trichinosis. *Proc. Helminthol. Soc. Washington.* **23**, 49-58.

Clapham, P. A. 1933. *Recent Researches on Helminth Immunity.* Imperial Bur. Agric. Parasitol., St. Albans, England, 1-20.

Cort, W. W. 1932. Variations in hookworm disease. *J. Parasitol.* **19**, 142-147.

Cort, W. W., and Otto, G. F. 1940. Immunity in hookworm disease. *Rev. Gastroenterol.*, **7**, 2-10.

Crofton, H. D. 1955. Nematode parasite populations in sheep on lowland farms. II. Worm egg counts in lambs. *Parasitology* **45**, 98-115.

Culbertson, J. T. 1938. Recent contributions to the immunology of helminthic infections. *Arch. Pathol.* **25**, 85-117; 256-280.

Ducas, R. 1921. *L'immunité dans la trichinose.* Thèse. Jouve et Cie., Paris, 47 pp.

Faust, E. C., and Meleney, H. E. 1924. Studies on schistosomiasis japonica. *Amer. J. Hyg. Monog.* **3**. Baltimore.

Fourie, P. J. J. 1931. The haematology and pathology of haemonchosis in sheep. *Union of South Africa Dept. Agric. Dir. Vet. Serv. and Animal Indust. Rept.* **17**, 495-572.

Fujinami, A. 1916. Kann die erworbene Immunität bei makroparasitärer Erkrankung vorkommen? *Kyoto Igaku Zassi* **13**, 31-33. (Japanese text, 176-185). (English Abstract: 1917, *China Med. J.* **31**, 81).

Fülleborn, F. 1929. Epidemiological observations on hookworm infection. Discusssion of the question of immunity and specific reactions of the host in helminthic infection. *British Med. J.* **1**, 755-759.

Fülleborn, F., Dios, R. L., and Zuccarini, J. A. 1928. Bericht über eine im Auftrage der argentinischen Regierung unternommene Reise nach der Provinz Corrientes und nach Paraguay zum Studium der Hakenwurmbekämpfung. *Arch. Schiffs.- u. Tropenhyg.* **32**, 441-481.

Gibson, T. E. 1952. The development of acquired resistance by sheep to infestation with the nematode *Trichostrongylus axie. J. Helminthol.* **26**, 43-53.

Glaser, R. W., and Stoll, N. R. 1938. Development under sterile conditions of the sheep stomach worm *Haemonchus contortus* (Nematoda). Science **87**, 259-260.

Glaser, R. W., and Stoll, N. R. 1940. Exsheathing and sterilizing infective nematode larvae. *J. Parasitol.* **26**, 87-94.

Goldberg, A. 1951. Life history of *Oesophagostomum venulosum*, a nematode parasite of sheep and goats. *Proc. Helminthol. Soc. Washington* **18**, 36-47.

Gordon, H. McL. 1948. The epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep. *Austral. Vet.* **24**, 17-44.

Gordon, H. McL. 1949. Epidemiology and the efficient parasite. *Rept. Austral. and N. Z. Assoc. Adv. Sci.*, 27th Meeting, pp. 131-141.

Gordon, H. McL. 1950. Some aspects of parasitic gastro-enteritis of sheep. *Austral. Vet. J.* **26**, 14-28; 46-52; 65-72; 93-98.

Hawkins, P. A., and Cole, C. L. 1945. Studies of sheep parasites. V. Immunity to gastro-intestinal nematodes. *J. Parasitol.* **31**, 113-118.

Hawkins, P. A., Cole, C. L., Kline, E. E., and Drudge, J. H. 1944. Studies of sheep parasites. I. The course of untreated nematode infections. *Vet. Med.* **39**, 154-161.

Herlich, H. 1954. The life history of *Nematodirus helvetianus* May, 1920, a nematode parasitic in cattle. *J. Parasitol.* **40**, 60-70.

Herrick, C. A. 1928. A quantitative study of infections with *Ancylostoma caninum* in dogs. *Am. J. Hyg.* **8**, 125-157.

Kates, K. C., and Turner, J. H. 1953. Experimental studies on the pathogenicity of *Nematodirus spathiger*, a trichostrongylid parasite of sheep. *Am. J. Vet. Res.* **14**, 72-81.

Kauzal, G. 1933. Seasonal incidence of gastro-intestinal parasites of fat sheep in New South Wales. *Austral. Vet. J.* **9**, 179,186.

Kauzal, G. 1937. A preliminary study of the pathogenic effect of Nematodirus spp. in sheep. *Austral. Vet. J.* 13, 120-123.

Levine, N. D., Bradley, R. E., Clark, D. T., and Kantor, S. 1956. The relation of semi-weekly pasture rotation to acquisition of gastrointestinal nematodes by sheep. *J. Parasitol.* 42, Suppl., 15.

Lucker, J. T., and Neumayer, E. M. 1946. Experiments on the pathogenicity of hookworm (*Bunostomum trigonocephalum*) infections in lambs fed an adequate diet. *Am. J. Vet. Res.* 7, 101-122.

McCoy, O. R. 1935. Artificial immunization of rats against *Trichinella spiralis*. *Am. J. Hyg.* 21, 200-213.

Marsh, H. 1936. Observations based on weekly parasite egg counts on feces of lambs and yearling sheep. *J. Parasitol.* 22,379-385.

Martin, C. J., and Ross, I. C. 1934. A minimal computation of the amount of blood removed daily by *Haemonchus contortus*. *J. Helminthol.* 12, 137-142.

Mayhew, R. L. 1940. Studies on bovine gastro-intestinal parasites. II. Immunity to hookworm and nodular worm infection in calves. *J. Parasitol.* 26, 345-357.

Mayhew, R. L. 1941. V. Immunity to the stomach worm, with a note on the prepatent period. *Am. J. Hyg.* 33*D*, 103-111.

Mayhew, R. L. 1944. VII. Attempts to develop an active immunity to *Haemonchus contortus* by injection of a saline extract of adult worms. *Proc. Helminthol. Soc. Washington* 11, 43-45.

Mayhew, R. L. 1948. X. The effects of nodular worm (*Oesophagostomum radiatum*) on calves during the prepatent period. *Am. J. Vet. Res.* 9, 30-34.

Mayhew, R. L. 1949a. XII. Additional infection experiments with the hookworm (*Bunostomum phlebotomum*) in the calf. *J. Parasitol.* 35, 315-321.

Mayhew, R. L. 1949b. XIV. *Haemonchus contortus* immunity experiments with injections of dried worm powder and immune blood serum. *Proc. Helminthol. Soc. Washington* 16, 19-23.

Mayhew, R. L. 1953. The parasites and parasitic diseases of cattle. *La. State Univ. and A. and M. Coll. Exper. Sta. Bull.* 428, 1-46.

Mönnig, H. O. 1927. The life-histories of *Trichostrongylus instabilis* and *T. rugatus* of sheep in South Africa. 11th-12th *Rept. Dir. Vet. Educ. and Res.*, Part 1, 231-251.

Morgan, D. O., Parnell, I. W., and Rayski, C. 1951. The seasonal variations in the worm burden of Scottish hill sheep. *J. Helminthol.* 25, 177-212.

Otto, G. F. 1948. Immunity against canine hookworm disease. *Vet. Med.* 43, 180-191.

Otto, G. F., Kerr, K. B., and Landsberg, J. W. 1937. The immunity to the hookworm, *Ancylostoma caninum*, acquired by dogs as a result of previous infection. *J. Parasitol.* 23, 560.

Porter, D. A. 1941. The effect of milk diet on the development of *Haemonchus contortus* in calves. *J. Parasitol.* 27, Suppl., 18-19.

Porter, D. A., and Cauthen, G. E. 1946. Experimental infection of calves with the small stomach worm, *Ostertagia ostertagi*. *J. Parasitol.* 32, Suppl., 14-15.

Roberts, F. H. S., O'Sullivan, P. J., and Riek, R. F. 1952. The epidemiology of parasitic gastro-enteritis of cattle. *Austral. J. Agric. Res.* 3, 187-226

Roberts, F. H. S., Turner, H. N., and McKevett, M. 1954. On the specific distinctness of the ovine and bovine "strains" of *Haemonchus contortus* (Rudolphi) Cobb (Nematoda: Trichostrongylidae). *Austral. J. Zool.* 2, 275-295.

Ross, I. C. 1931. The host specificity of *Haemonchus contortus* of sheep and cattle. *Austral. J. Exp. Biol. & Med. Sci.* 8, 217-224.

Ross, I. C. 1932. Observations on the resistance of sheep to infestation by the stomach worm (*Haemonchus contortus*). *J. Council. Sci. & Indust. Res.* 5, 73-80.

Ross, I. C., and Kauzal, G. 1933. Preliminary note on the pathogenic importance of *Chabertia ovina* (Fabricius, 1788). *Austral. Vet. J.* 9, 215-218.

Sandground, J. H. 1928. Some studies on susceptibility, resistance, and acquired immunity to infection with *Strongyloides stercoralis* (Nematoda) in dogs and cats. *Am. J. Hyg.* 8, 507-538.

Sandground, J. H. 1929. A consideration of the relation of host-specificity of helminths and other metazoan parasites to the phenomena of age resistance and acquired immunity. *Parasitology.* **21,** 227-255.

Sawyer, W. A. 1925. Factors that influence the rate of increase of hookworm infection. *Am. J. Hyg.* **5,** 790-817.

Sarles, M. P. 1932. Development of an acquired resistance in rabbits by repeated infection with an intestinal nematode, *Trichostrongylus calcaratus* Ransom, 1911. *J. Parasitol.* **19,** 61-82.

Sarles, M. P. 1938. The *in vitro* action of immune rat serum on the nematode, *Nippostrongylus muris. J. Infect. Dis.* **62,** 337-348.

Seddon, H. R. 1931. The development in sheep of immunity to *Moniezia expansa. Ann. Trop. Med. & Parasitol.* **25,** 431-435.

Sheldon, A. J. 1937. Studies on active acquired resistance, natural and artificial, in the rat to infection with *Strongyloides ratti. Am. J. Hyg.* **25,** 53-65.

Sheldon, A. J., and Groover, M. E., Jr. 1942. An experimental approach to the problem of acquired immunity in human hookworm (*Necator americanus*) infections. *Am. J. Hyg.* **36,** 183-186.

Smith, T., and Ring, E. R. 1927. The segregation of lambs at birth and the feeding of cow's milk in the elimination of parasites. *J. Parasitol.* **13,** 260-269.

Spindler, L. A. 1937. Resistance to intestinal trichinosis in experimental animals induced by feeding metabolic products of encysted trichinae. *J. Washington Acad. Sci.* **27,** 36-38.

Sprent, J. F. A. 1946. Immunological phenomena in the calf, following experimental infection with *Bunostomum phlebotomum. J. Comp. Pathol. & Therap.* **56,** 286-297.

Stewart, D. F. 1950a. Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* spp. and on the immunological reactions of sheep exposed to infestation. I. The preparation of antigens for the complement fixation test and the reactivity of the biochemical fractions of *H. contortus. Austral. J. Agric. Res.* **1,** 285-300.

Stewart, D. F. 1950 b. II. The antibody response to infestation with *H. contortus. Austral. J. Agric. Res.* **1,** 301-321.

Stewart, D. F. 1950 c. III. The antibody response to infestation with *Trichostrongylus* spp. *Austral. J. Agric. Res.* **1,** 413-426.

Stewart, D. F. 1950 d. IV. The antibody response to natural infestation in grazing sheep and the "self-cure" phenomenon. *Austral. J. Agric. Res.* **1,** 427-439.

Stewart, D. F. 1953. V. The nature of the "self-cure" phenomenon. *Austral. J. Agric. Res.* **4,** 100-117.

Stewart, D. F., and Gordon, H. McL. 1953. VI. The influence of age and nutrition on resistance to *Trichostrongylus colubriformis. Austral. J. Agric. Res.* **4,** 340-348.

Stewart, D. F., 1955. "Self-cure" in nematode infestations of sheep. *Nature.* **176,** 1273-1274.

Stewart, M. A., Miller, R. F., and Douglas, J. R. 1937. Resistance of sheep of different breeds to infestation by *Ostertagia circumcincta. J. Agric. Res.* **55,** 923-930.

Stoll, N. R. 1928. The occurrence of self-cure and protection in typical nematode parasitism. *J. Parasitol.* **15,** 147-148.

Stoll, N. R. 1929 a. [Experimental infections with the twisted wire worm, *Haemonchus contortus,* in sheep.] *J. Parasitol.* **15,** 217 (*Proc. Helminthol. Soc. Washington,* 111th Meeting, March 17, 1928).

Stoll, N. R. 1929 b. Studies with the strongyloid nematode, *Haemonchus contortus.* I. Acquired resistance of hosts under natural reinfection conditions out-of-doors. *Am. J. Hyg.* **10,** 384-418.

Stoll, N. R. 1930. On methods of counting nematode ova in sheep dung. *Parasitology* **22,** 116-136.

Stoll, N. R. 1932. Note on re-infection under "natural" conditions with a gut nematode of the rabbit. *J. Parasitol.* **19,** 54-60.

Stoll, N. R. 1936 a. Certain net effects in helminthic parasitism, with special reference to the sheep host. *Cornell Vet.* **36,** 171-179.

Stoll, N. R. 1936 b. Observations on cattle nematode infections, with a demonstration of their secondary transmission to grazing sheep. *J. Parasitol.* **22,** 386-407.

Stoll, N. R. 1940 a. Reinfection of grazing sheep with gastro-intestinal nematodes,—a synoptic experiment, with follow-up. *3rd Inter. Congr. Microbiology,* New York, Sept. 1939, *Rept. Proc.* 185-186.

Stoll, N. R. 1940 b. Worm-host systems as labile mechanisms: A view of the nematode-ruminant problem. *J. Am. Vet. Med. Assoc.* **96,** 305-308.

Stoll, N. R. 1942. Active immunization of sheep against large single test infections of *Haemonchus contortus. J. Parasitol.* **28,** Suppl, 20.

Stoll, N. R. 1943. The wandering of *Haemonchus* in the sheep host. *J. Parasitol.* **29,** 407-416.

Stoll, N. R. 1947. This wormy world. *J. Parasitol.* **33,** 1-18.

Swales, W. E. 1940. The helminth parasites and parasitic diseases of sheep in Canada. I. A survey and some preliminary studies on existing problems. *Canadian J. Res.* **18,** 29-48.

Taliaferro, W. H. 1929. *The Immunology of Parasitic Infections.* Century, N.Y.

Taliaferro, W. H. 1940. The mechanism of acquired immunity in infections with parasitic worms. *Physiol. Rev.* **20,** 469-492.

Taliaferro, W. H. 1943. Antigen-antibody reactions in immunity to metazoan parasites. *Proc. Inst. Med. Chicago* **14,** 358-368.

Taylor, E. L. 1934 a. Field experiments on the immunity of lambs to parasitic gastritis caused by a mixed infection of trichostrongylid nematodes. *J. Helminthol.* **12,** 143-164.

Taylor, E. L. 1934 b. The epidemiology of winter outbreaks of parasitic gastritis in sheep. *J. Comp. Pathol. & Therap.* **47,** 235-254.

Tetley, J. H. 1935. Ecological studies on *Nematodirus* species in sheep in Manawatu district, New Zealand. *J. Helminthol.* **13,** 41-58.

Tetley, J. H. 1949. Rhythms in nematode parasitism of sheep. *N. Z. Dept. Sci. & Indust. Res., Bull.* **96,** 1-214.

Thorson, R. E. 1953. Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris. Am. J. Hyg.* **58,** 1-15.

Thorson, R. E. 1954. Absorption of protective antibodies from serum of rats immune to the nematode, *Nippostrongylus muris. J. Parasitol.* **40,** 300-303.

Threlkeld, W. L. 1948. The life history and pathogenicity of *Chabertia ovina* (Fabricius, 1788) Railliet and Henry, 1909. *Va. Agric. Exper. Sta. Tech. Bull.* **111,** 1-27.

Threlkeld, W. L., and Downing, T. O. 1936. A report on experimental infections of *Ovis aries* with the infective larvae of *Ostertagia circumcincta. J. Parasitol.* **22,** 187-201.

Turner. J. H. 1956. Further studies on strongyloidiasis in lambs and kids: Development of acquired resistance. *J. Parasitol.* **42,** Suppl., 18-19.

Vegors, H. H. 1954. Experimental infection of calves with *Strongyloides papillosus* (Nematoda). *Am. J. Vet. Res.* **15,** 429-433.

Watt, J. Y. C. 1943. Active immunization of rats against *Nippostrongylus muris. Proc. Soc. Exp. Biol. & Med.* **52,** 67-72.

Wetzel, R. 1952. Helminthen und Immunität. *Zentralb. Bakt., Parasitenk., Infektionskr. u. Hyg.* I. Orig. **158,** 199-205.

Whitlock, J. H. 1949. The relationship of nutrition to the development of the strongylidoses. *Cornell Vet.* **39,** 146-182.

Whitlock, J. H. 1955. A study of the inheritance of resistance to trichostrongylidae in sheep. *Cornell Vet.* **45,** 422-439.